DERIVATISATION OF NERVE AGENT DEGRADANTS WITHOUT REMOVAL OF WATER

By

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DECLARATION

I declare that this manuscript does not contain any material submitted previously for the award of any other degree or diploma at any university or other tertiary institution. Furthermore, to the best of my knowledge, it does not contain any material previously published or written by another individual, except where due references has been made in the text. Finally, I declare that all reported experimentations performed in this research were carried out by myself, except that any contribution by others, with whom I have worked is explicitly acknowledged.

Signed: VANCE LANG TIEN NGUYEN

Dated: **30/06/19**

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LIST OF ABBREVIATIONS

CWA	Chemical Warfare Agents
CWC	Chemical Weapons Convention
OPCW	Organisation for Prohibition of Chemical Weapons
APA	Alkylphosphonic Acid
MPA	Methylphosphonic Acid
GC–MS	Gas Chromatography–Mass Spectroscopy
MTBSTFA	N-Methyl-N-tert-butyldimethylsiyltrifluoroacetamide
LOD	Limit of Detection
LC–MS	Liquid Chromatography–Mass Spectroscopy
IMPA	Isopropyl Methylphosphonic Acid
EMPA	Ethyl Methylphosphonic Acid
NMR	Nuclear Magnetic Resonance
FPD	Flame Photometric Detection
EI	Electron Ionization
CI	Chemical Ionization
TMS	Trimethylsilylation
GC–MS/MS	Gas Chromatography–Tandem Mass Spectroscopy
LC–MS/MS	Liquid Chromatography–Tandem Mass Spectroscopy
PFBBr	Pentafluorobenzylbromide
ESI	Electrospray Ionization
IS	Ionspray
APCI	Atmospheric Pressure Chemical Ionization
TBDMS	Tert-butylmethylsilylation
BSTFA	*N,O*-bis (trimethylsilyl)trifluoroacetamide

(TBDMSCl)	Tert-butyldimethylsilyl chloride
HCl	Hydrochloric Acid
2-DMAMP	2-[(dimethylamino)methyl]phenol

ABSTRACT

Detection of methylphosphonic acid is used as a marker for potential contamination of organophosphorus nerve agents in the environment. Analysis of this compound is difficult and time-consuming due to the requirement of derivatisation in order to make the compound suitable for GC-MS. A pilot study has found success in derivatizing methylphosphonic acid without requiring the elimination of water however, the efficiency of this method is rather low from the following quantitative study. The reliability of the quantitative study however, is questioned due to the deteriorating concentration over time despite sources stating that methylphosphonic acid is very stable. Irreversible adsorption of the compound onto laboratory equipment was the proposed reasoning behind this observation. Assessing the effects of this phenomenon and evaluating methods to minimise this issue will assist in the development of a more effective and efficient procedure to analyse methylphosphonic acid chromatically.

1.0 Introduction

Development, production, and use of chemical warfare agents (CWA) have been prohibited since the signing of the treaty by over 170 countries at the Chemical Weapons Convention (CWC). CWC formed the Organisation for Prohibition of Chemical Weapons (OPCW) in 1997 which now administers this treaty and inspects countries to ensure that the treaty is being withheld [1,2,17]. Despite these efforts, CWA is still being developed and are used in terrorist attacks and wars to this day [2].

CWA however, are not very persistent in environmental conditions thus making it difficult to detect. It then becomes more important to be able to identify and detect their degradation products as they are good markers for their parent compound. Organophosphorus nerve agents such as Sarin and Soman, undergoes rapid hydrolysis in aqueous environments to form alkylphosphonic acids (APA) [3,4]. This chemical undergoes further hydrolysis to form methylphosphonic acid (MPA) [24]. MPA is very persistent and does not readily undergo any further degradation. MPA is not a naturally occurring chemical and has minimums uses which makes it ideal for determining if organophosphorus nerve agents were present [5].

Gas Chromatography–Mass spectroscopy (GC-MS) is the most widely used method for the identification of CWA degradants [2]. MPA however, is non – volatile and highly polar which makes it unsuitable for this technique [6]. MPA can undergo derivatisation which alters it's properties and allows it to be more suitable for GC – MS [7]. Derivatisation however, requires the elimination of water which is a difficult process and causes more potential sources of errors [5].

In 2018, Dival attempted to derivatize MPA without the need to remove water. Dival successfully derivatised MPA using N-Methyl-N-tert-butyldimethylsiyltrifluoroacetamide (MTBSTFA) with the addition of hexane in order to create a two-phase solution. The derivative was then able to be detected in the organic layer using GC-MS with a limit of detection (LOD) of 1000ppm [8]. A quantitative study was then followed up Chua to assess the efficiency of the reaction by measuring the amount of MPA in the aqueous layer using liquid chromatography–mass spectroscopy (LC-MS). Chua had concluded that the efficiency of the two-phase derivatisation was 14.5% [9]. During the experiment, Chua commented that the concentration of MPA in the calibration standards were declining over time which led to very inconsistent results. A possible explanation for this degradation was that MPA had irreversibly adsorbed on to the surface of the laboratory glassware.

Adsorption is the phenomenon in which a substance can accumulate and adhere to a surface [10]. Irreversible adsorption to glassware can occur due to the substance reacting to silanol groups found on the surface via ionic exchange [11]. Irreversible adsorption is a prevalent issue when dealing trace amounts as loss of the sample can jeopardize the reliability of the results [12,13]. This review aims to discuss the effects of adsorption that occurs on laboratory equipment in order to develop a solution to minimise this phenomenon on the analysis of MPA. This would assist in improving the reliability of the quantitative study in the development of a more efficient method in the detection and identification of methylphosphonic acid.

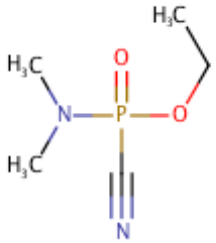
2.0 Organophosphorus Nerve Agents

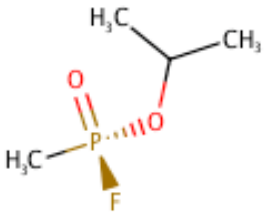
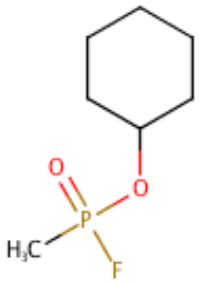
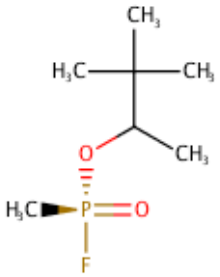
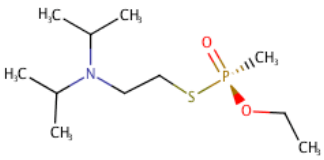
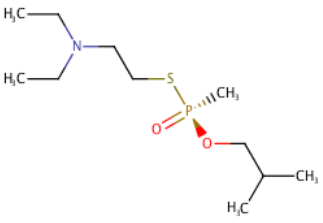
Chemical warfare agents have become a serious issue since it was first used in World War I [14]. Among them, agents made from organophosphorus compounds are the deadliest forms of CWAs [1,14,15]. Also known as "nerve agents", these compounds are anticholinesterases, which inhibits the enzyme acetylcholinesterase which prevents the degradation of acetylcholine at the neuronal synapse and neuromuscular junctions [1,14,16]. This is caused by a covalent P-O bond forming at the serine hydroxyl group on the enzyme [16]. A build-up of excess acetylcholine causes an over stimulation of the cholinergic receptors also known as a "cholinergic crisis" which leads to seizures, respiratory failure, muscle spasms and death [15,16].

2.1 Types of Nerve Agents

Nerve Agents are categorised into 2 different groups. "G" nerve agents such as Sarin (GB) and Tabun (GA) originated from Germany hence the letter G. The other group known as the "V" series which stands for venomous, are used to identify agents such as "VX" which are more toxic than the "G" series nerve agents.

Table 1: Common Organophosphorus Nerve Agents [3,4]

Nerve Agent **CAS number**	**Chemical Name** **Chemical formula**	**Structure**
Tabun (GA) 77-81-6	Ethyl dimethylphosphoramido-cyanidate $C_5H_{11}N_2O_2P$	

Sarin (GB) 107-44-8	Isopropyl methylphosphonofluoridate $C_4H_{10}FO_2P$	
Cyclosarin (GF) 74192-15-7	Cyclohexyl methylphosphonofluoridate $C_7H_{16}FO_2P$	
Soman (GD) 96-64-0	Pinacolyl methylphosphonofluoridate $C_7H_{14}FO_2P$	
VX 50782-69-9	O-ethyl S-2-diisopropylaminoethyl methylphosphonothiolate $C_7H_{18}NO_2PS$	
Russian VX (VR) 505-60-2	S-(N,N-diethylaminoethyl) isobutyl Methylphosphonothiolate $C_{11}H_{26}NO_2PS$	

V series agents are not as volatile compared to the G series agents and will persist in the environment far longer than the G series [3,17].

2.2 Brief History of Organophosphorus Nerve agents

Organophosphorus compounds were mainly used as pesticides prior to the development of CWAs. Organophosphorus pesticides function similarly to that of its warfare counterpart as they both function as anticholinesterases. It is difficult to determine the exact origins of organophosphorus nerve agents, but reports indicate that both Sarin and Tabun were manufactured in Germany by Gerhard Schrader in 1937 [1,14,18]. Use of nerve agents (Sarin and Tabun) however, was first used in the Persian Gulf War by Iraq against Iraq which occurred in 1980 and ended in 1988 [17]. A terrorist attack involving Sarin was conducted by the Japanese cult Aum Shinrikyo in which left 7 dead in Matsumoto in 1994, 13 dead in Tokyo in 1995 with 5500 injured as well [17,18]. A more recent use of nerve agents was the discovery of Novichok that was mysteriously deployed in the United Kingdom in 2018 [19].

2.3 Nerve Agent Degradants

Organophosphorus nerve agents are alkyl phosphonic acid esters [3]. All nerve agent's in both G and V series contain a C – P bond that is not found in organophosphate pesticides. Nerve agents are quite volatile and will rapidly degrade to alkyl phosphonic acids via hydrolysis between phosphorus atoms and a leaving group within the compound [3,4]. The rate at which these nerve agents decompose depends heavily on the temperature, conditions of the environment, volatility and solubility of the agent in water. The C – P bond is very persistent and is present even during degradation [3]. This C – P bond allows these degradants to become markers in the determination of nerve agent contamination in the environment.

APAs can hydrolyse further to form more stable compounds. Sarin (GB) is the most volatile of the G agents and undergoes hydrolysis by loss of fluoride to form isopropyl methylphosphonic acid (IMPA) and hydrofluoric acid [3]. IMPA only has a reported half life of approximately 8 to 13 days before degrading into MPA via hydrolysis [3,20].

Like Sarin, Soman (GD) hydrolyses to form pinacolyl methylphosphonic acid losing the fluoride group to form hydrofluoric acid. Soman hydrolyses at a slower rate compared to Sarin due to the alkoxy group that is present. This functional group is eventually lost to form MPA.

Tabun (GA) under neutral conditions degrades to 0-ethyl N,N dimethylamido phosphoric acid, losing its cyanide group in the process. Dimethylphosphoriamidate can be formed due to further hydrolysis which will then slowly degrade to phosphoric acid. It is reported that theoretically, Tabun can also hydrolyse to form MPA however the likely hood of detecting MPA that originated from Tabun is slim [3,4,20]. Tabun under acidic conditions will degrade to form ethylphosphoryl cyanidate.

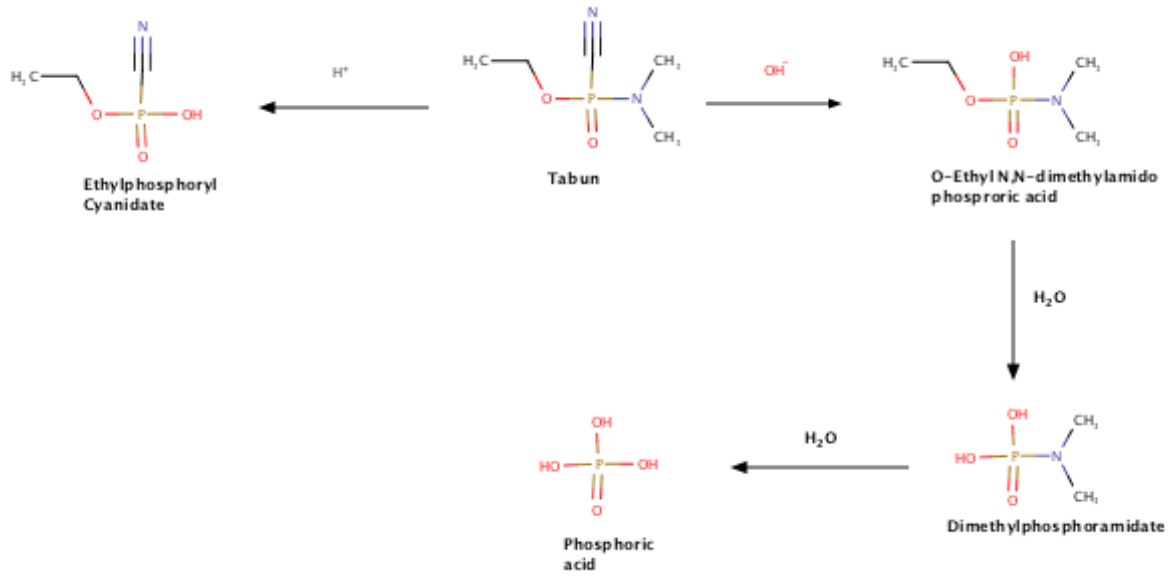


Figure 1: Degradation pathways for Tabun under acidic or basic conditions. Adapted and modified from from Munro N. [3].

V series nerve agents are not as volatile compared to the G series and are quite persistent on the surface [4]. VX is soluble in water however, is relatively more resistant to hydrolysis compared to its G counterparts. It is reported that in water that is at room temperature with neutral conditions, that half life of VX ranges from 17 – 42 days [3]. Despite this, VX will slowly degrade and will hydrolyse down two separate pathways which are dependent on the environmental conditions. In environments where the pH is less than 7 and greater than 10, the P – S bond is cleaved to form both ethyl methylphosphonic acid and Diisopropyl ethyl mercraptoamine. Ethyl methylphosphonic acid (EMPA) like other AMPAs further decomposes to MPA. MPA cannot be formed where EMPA is found in aqueous conditions however, there are reports of MPA being found in soil that has been contaminated with VX [4]. In environments with pH levels that are between 7 and 10, the C – O bond from the ethoxy group is instead cleaved to form S- (2-Diiosproylaminoethyl) methyl phosphonothioate and ethanol.

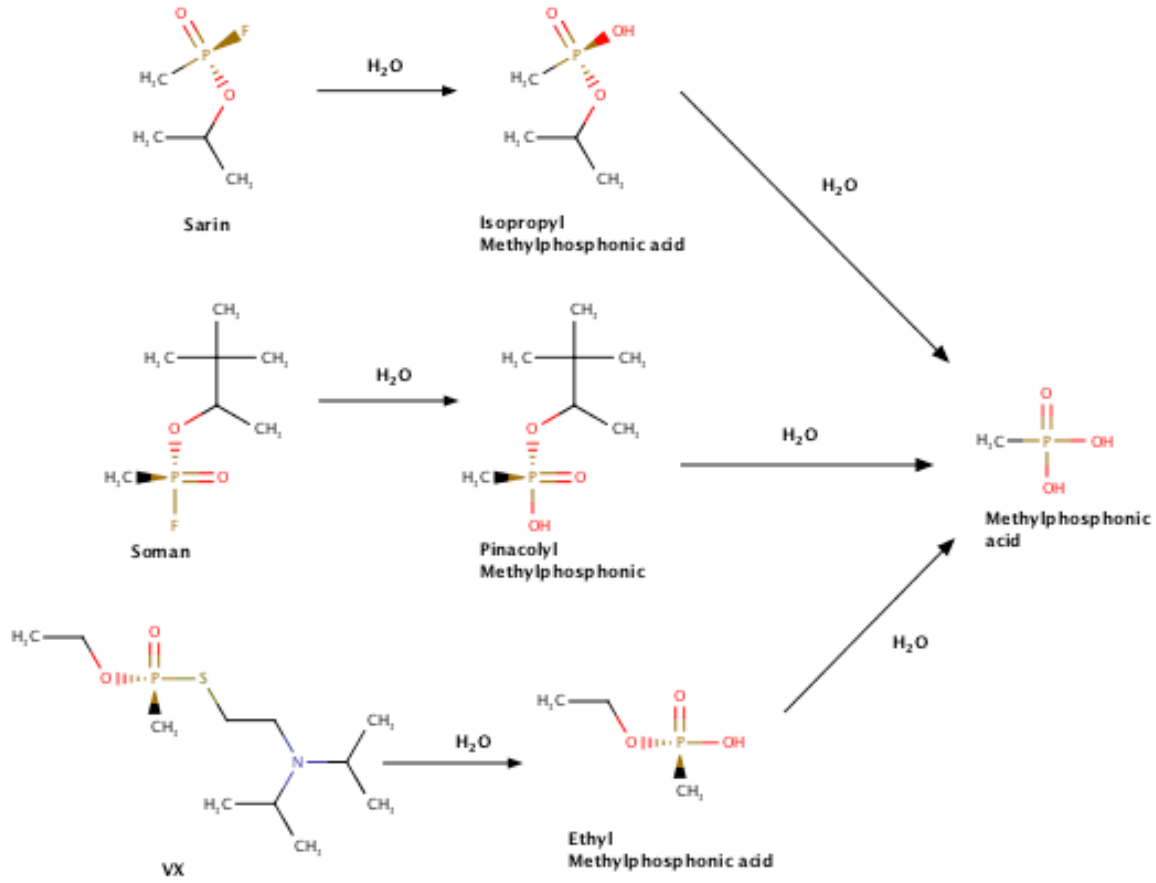


Figure 2: Hydrolysis Pathways for Sarin, Soman ,and VX. Adapted and modified from Munro N. and Seto Y. [3,7]

Due to their stability and persistence, organophosphorus hydrolysis products become valuable markers for contamination of nerve agents. MPA is a potential product in most nerve agents that degrade to form APAs. MPA is the most stable product from these reactions with a half life reported up to 18 years in water [36]. MPA not being naturally occurring in the environment and having limited uses makes it ideal for determination of nerve agents. Black has reported that MPA is a degradation product to some fire retardants but without a reference to another source, this can't be proven [5,15,28]. There is also a possibility that MPA or other methyl phosphorus compounds can be found in trace amounts as industrial waste in big cities [21]. Being able to detect this compound becomes crucial to the determination of several organophosphorus nerve agents. The only exception however is

Tabun which mainly hydrolyses into phosphoric acid. MPA can also possibly degrade into phosphoric acid but due to the stability of MPA, the probability of this occurring is slim[60]. Phosphoric acid degrades into phosphorus which has more uses compared to MPA such as being a major chemical in pesticides and plasticisers [5].

3.0 Analysis methods for Degradants

The OPCW requires that unambiguous identification of chemical warfare agents and its degradants must be conducted using two or more different spectrometric techniques and references [22]. The most common techniques used to analyse warfare agents today are gas chromatography–mass spectroscopy (GC-MS) and liquid chromatography–mass spectroscopy (LC-MS).

3.1 **Gas Chromatography–Mass Spectroscopy**

Due to the volatility of chemical warfare agents, Gas chromatography was the most effectively used method due to its high efficiency and sensitive detection [2]. Nerve agents also contain a phosphorus atom which makes it highly suitable for detection using flame photometric detection (FPD) or nitrogen-phosphorus detection (NPD). GC measures a compounds retention indices which are then compared to known standards for identification. Minami was able to detect MPA using trimethylsilylation (TMS) derivatisation from urine using GC-FPD with a detection limit of 0.625μm [18]. Nakajima was also able to detect MPA in urine using a similar method to Minami's but opted to use MTBSTFA as the derivatising reagent [26]. Due to the current requirement for spectrometric results by the CWC, the use of GC alone has decreased in favour of GC-MS which has become more readily available [2].

GC-MS is the most popular method in determining and identifying nerve agents and their degradation products [2,18]. Using GC-MS both structural information and molecular mass can be obtained from a sample. Structural information can be obtained using electron ionization (EI) while molecular mass can be obtained using chemical ionisation (CI) [2]. OPCW requires results from both EI and CI as CI is used to confirm the results of EI [25].

Riches provided a generic GC-MS method for the analysis of organophosphorus compound [68]:

Table 2: Generic GC-MS Method for Nerve Agents. Adapted from Riches J. [68]

	Properties / Parameters	Suggested Method
GC	Column	25–30 m, 0.20–0.25 mm i.d. (0.25–0.33 μm film thickness), 95% methyl–5% phenyl polysiloxane low bleed column
	Injection Mode	Splitless
	Injection Volume	1μL
	Splitless Time	Up to 1 min
	Injector Temperature	200-280°C
	Carrier Gas	Helium
	Septum Purge Flow	2-4ml/min
	Temperature Programme	40 °C (1 min) to 280 °C at a rate of 10 °C/min (hold for 5–10 min)
MS	Solvent Delay	3 min
	Mass Range	m/z 40-550
	Scan Rate	>1scan/s
	Electron Energy	70 eV

GC-MS however, is not a suitable technique for "pure" MPA as the compound is highly polar and non-volatile [24]. GC-MS analysis can only be conducted on MPA after it has undergone derivatisation in order to make it more suitable for the technique. GC-MS has proven to be able to detect derivatized MPA in many different situations. Tripathi was able to detect MPA

4 weeks after it had been synthesized and mentions that MPA was still present for up to 12 weeks. Diazomethane was used as the derivatising agent and the product gave rise to a major peak at m/z 96 and a minor peak m/z 97 with a concentration of 10μg MPA in 100ml of water [24].

GC-MS has also been used to detect MPA in blood plasma and urine. Kataoka attempts to deproteinize plasma using acetonitrile in order to minimise the effect that proteins will have on GC-MS. MPA was then derivatized using TBDMS and then analysed using GC-MS but provided only an 8% yield [63]. A second method that used trichloroacetic acid for de-proteination was able to achieve a detection of yield of 61-97% of the TBDMS derivative [63].

Rohrbaugh was able to detect TMS derivatives of APAs using Gas chromatography –tandem mass spectroscopy (GC-MS/MS) [23]. Ethyl, isopropyl, isobutyl, pinacolyl and cyclohexyl – MPA was able to be detected in diesel fuel and BNA-pesticide by monitoring the dissociation of the m/z 153 parent ion to the m/z 75 ion under EI conditions [23]. Ammonia CI outperformed methane CI due to the later not providing accurate molecular mass measurements for larger alkyl groups. Although MPA was not directly tested, analysis using this method should theoretically work with MPA due to the similar structure (OH group instead of alkyl). Use of methane CI can be implemented as well due to the absence of an alkyl group.

3.2 Liquid Chromatography – Mass Spectroscopy

Nerve agent degradants can also be analysed using LC-MS. LC-MS is a separation technique that compared to GC-MS, is very suited for the determination of polar and non-volatile compounds such as MPA. Mass spectrometry using liquid chromatography for the analysis of hydrolysis products is often conducted using electrospray ionization (ESI) although ionspray

(IS) and atmospheric pressure chemical ionization (APCI) can both be used as complementary methods [27]. Both positive and negative ion modes have been used with success in both methods. MPA fragments to form $[M-H]^-$ ions at m/z 95 in negative mode and $[M+H]^+$ ions at m/z 97 in positive mode.

Riches similar to GC-MS has also provided a generic LC-MS method for organophosphorus compounds [68]:

Table 3: Generic LC-MS Method for Oraganophosphorus Compounds. Adapted from Riches J. [68]

	Properties / Parameters	**Suggested Method**
LC	Column	150 by 2.0 mm C18
	Mobile Phase Gradient	**APCI** A: 20 mM ammonium formate in water B: 20 mM ammonium formate in methanol **ESI** A: 0.1% formic acid in water B: 0.1% formic in acetonitrile
	Mobile-phase flow rate	200µL/ min
	Mobile-phase gradient	5% B (0-5 min) to 90% B (15min). Hold at 90% B (5min)
MS	Source Conditions	**APCI** Vaporiser Temp: 400 °C Corona current: 4-6 µA **ESI** ESI Voltage: 3-5kV

Source Condition Induced Dissociation	5-25 V
Mass Range	m/z 40-400
Scan Rate	1 scan/s
Sheath, Sweep and Auxillary gas	Nitrogen

Read and Black have reported better LODs using positive ion spray compared to negative ion spray for detecting MPA. ESI also performed better than APCI having a detection limit of <50ng/ml [30]. APCI although less sensitive, is found to be more robust in a follow-up study conducted my Read and Black and is better suited for other CWA [29].

Mawhinney has found success in adding a mobile phase post column in order to increase the response of Alkyl-MPAs using liquid chromatography – tandem mass spectroscopy (LC-MS/MS). The addition aprotic solvents and large alcohols increased the number of ions introduced into the gas phase which has increased the signal intensity of the mass spectrometer [30]. Mawhinney has concluded that these additions increased the signal intensity and signal to noise ratio by factors of 60 and 19 [30].

Otsuka has had success detecting MPA using LC–MS/MS with a reverse phase column. Otsuka's method was able to perform more efficiently with better LODs (33ng) compared to conventional LC-MS/MS techniques and even GC-MS after MPA had been derivatised with pentafluorobenzylbromide (PFBBr) [4].

Weissberg has developed a method that extracts, derivatives and analyses using LC-MS, G-nerve agent in approximately 20 minutes. Nerve agents such as sarin, soman, and cyclosarin

as well as its hydrolysis products were able to be extracted from soil and other matrices in 2 minutes using water and 2-[(dimethylamino)methyl]phenol (2-DMAMP). 2-DMAMP also doubles up as the derivatizing agent. The derivatives were then analysed using LC-ESI-MS in positive ion mode. The reported LOD of this method was 0.8-20pg/cm^2 in asphalt and concrete and 4pg/g in soil [64].

Baygildiev had developed a time-efficient protocol in 2017 for the determination of MPA using LC/MS/MS. Analysis was conducted using an Agilent 6460 Triple Quad LC/MS system. The method (outlined in table 2) was able to obtain a mass spectrum that contained a strong peak at m/z 95 which corresponds to the deprotonated MPA. The method had a LOD of 10ng/ml, limit of quantitation of 30ng/ml with good results with concentrations between 30-1000ng/ml [58]. The LOD recorded in this experiment is far lower than the suggested LOD of MPA from LC/MS which is thought to be 50ng/ml [58].

Table 4: Proposed Time-Efficient LC/MS/MS method, adapted from Baygildiev [58]

Properties / Parameters	Suggested Method
Stationary Phase	Acclain RSLC column (150 x 2.1mm; 2.2μm)
Mobile phase	A: 0.5% formic acid B: ACN With ratio 95:5
Delivered Rate	0.4ml/min
Temperature	Nebulizer gas: 350°C Sheath gas: 400°C
Gas Flow Rate	Nebulizer: 10 L/min

	Sheath: 11 L/min
Ionization Voltage	4500V

Although LC-MS is better suited for the analysis of MPA, GC-MS is preferred because of its unambiguous identification of MPA [30]. GC-MS provides better selectivity and sensitivity compared to LC-MS. LC-MS however provides rapid screening of aqueous samples without the requirement of derivatisation. LC-MS is best used as a complementary technique or if analysis via GC-MS can not be conducted.

4.0 Derivatisation

A derivatisation reaction converts a polar functional group to a non-polar group in order to make the chemical more suitable for GC-MS. Other than changing the polarity, derivatisation also alters other properties to make the sample more suited for chromatographic analysis. The reaction can be used to reduce the volatility and reactivity, in order to minimise the possibility of reacting to the instrument or evaporating in the air. Sensitivity is also increased allowing the samples to be detected using more sensitive techniques e.g. negative ion chemical ionisation mass spectroscopy [5]. Derivatisation allows better resolution of peaks during analysis via chromatography [5].

4.1 Common Reagents for MPA

MPA needs to be derivatised so that the compound becomes suitable for analysis via GC-MS. Black describes the ideal derivatising reagent to be one that allows a rapid derivatisation reaction that requires minimum energy input [31]. Reagents should also have good chromatographic properties, present minimal hazards and be commercially available [31].

The most common used derivatisation method for MPA is silylation such as TMS and TBDMS to form silyl esters. Silylation was used in the identification of Sarin in Japan [18,26]. *N,O*-bis (trimethylsilyl)trifluoroacetamide (BSTFA) are used to produce TMS derivatives. BSTFA with the addition of 1% trimethylsilyl chloride has been reported to have 80-100% derivatisation efficiency [32]. TBDMS esters are produced using MTBSTFA with or without the addition of catalyst in 1% tert-butyldimethylsilyl chloride (TBDMSCl). Use of a catalyst improves the stability of the derivative however Black argues that TBDMSCl provides lower yields and creates large amounts of by-products [9,31]. Compared to TMS esters, TBDMS products are more stable (up to 6 days without degradation) and less prone to

react with water [33,34]. Derivatisation of MPA using this method however, is not suitable if there is calcium or mercury present in the analyte. MPA derivatized from TBDMS was not able to be detected in soil samples that high concentrations of both calcium and mercury ions in a study conducted by Katoka [60]. OPCW has recommended the use of a cation-exchange resin in order to remove metal ions from aqueous extracts in order to minimise this issue [31,61].

APAs can also be derivatised using diazomethane to form methyl esters. Diazomethane is a very efficient reagent with recorded reaction yields of up to 99% [35]. Diazomethane is highly reactive with acidic compounds thus are able to produce methyl esters rapidly [22]. Diazomethane is also described to be highly toxic, potential for detonation and due to its high volatility needs to be freshly synthesized before use [31]. Despite being able to quickly and efficiently derivatise MPA, derivative products using this reagent have less than ideal chromatographic properties [31]. Black states that methyl esters give rise to poor peak shapes especially those derived from MPA which have short retention times thus increasing the chance for interference [31].

There has also been success in derivatising MPA using PFBBrs to form pentaflurobenzyl esters. Riches and Black states that this method coupled with negative ion chemical ionization provides the lowest LOD [31,62]. Riches was able to achieve a LOD of 0.1ng/ml for isopropyl, isobutyl, pinacolyl and cyclohexyl – MPAs that was recovered from urine [62]. The disadvantages of using this method however is that the method is slow and requires more complex conditions in order to achieve a successful reaction [31].

Weissberg has had success in derivatizing G-nerve agents using 2-DMAMP [64,65]. Derivatisation using this reagent can occur as fast as 1 minute and proceeds at ambient temperatures [65]. Derivatives are also reportedly stable for up to 48 hours. Using LC-ESI-MS/MS in positive ion mode, the reported LOD for this method was 1pg/ml [65]. 2 – DMAMP has also had success in extracting G-nerve agents in Weissbergs follow up study [64].

4.2 Disadvantages:

Although derivatisation does solve several issues to help improve the suitability for GC-MS, it does present some disadvantages that must be considered. The largest issue that derivatisation presents is that water cannot be present and needs to be evaporated from the sample to dryness [5,15,27-30]. If water is present even in trace amounts, there is a possibility that it reacts with the derivatising reagent or the derivative product itself which may modify the desired properties that are required for analysis [5]. Extraneous materials that are also present in the sample can minimise the efficiency of the reaction or can react with the derivatising agent to produce a complex background [5]. Despite research proving certain derivatisation methods perform better than others, on a whole derivatisation produces unstable products that are required be analysed as soon as possible. Attempting to evaporate the analyte to complete dryness is has also been proven to be very time consuming [27-30].

4.3 Current Studies:

Due to the complications that can occur when attempting to remove water from the sample, studies have been conducted to determine of derivatising without the need to remove water was possible. In 2018, a pilot study was conducted that suggested adding an organic layer into the reaction. Dival derivatised MPA using MTBSTFA with the addition of hexane. The

MPA derivative was able to be detected in the organic layer using GC-MS however the limit of detection was recorded to be 1000ppm [8]. Although the method was successful the limit of detection questions the practicality of this process. Literature suggests that the average concentration of MPA that can be expected to be found in the environment to be approximately 1-10ppm which is significantly smaller than recorded the limit of detection [22].

A quantitative study on this proposed method was conducted in 2018 by Chua. The goal of this study was to determine the efficiency of the two-phase derivatisation. Chua opted to use MTBSTFA with 1% (TBDMSCl) rather than pure MTBSTFA in order to produce a more hydrolytically stable derivative for analysis using LC-MS [9]. MPA was able to be successfully detected in the organic layer with the derivatising agent using GC-MS. Similar to Duval's experiment, MPA was only able to be successfully detected at 1000mg/L [9]. signals were detected on the chromatograms of smaller concentrations however there was not insufficient evidence to confirm the MPA – derivative.

A calibration curve was constructed using standards made from various concentrations of solid MPA dissolved in de-ionised water. When analysing the standards using LC-MS however, Chua noticed inconsistent peak areas from each calibration standard. Despite multiple repetitions, the peak area severely increased despite having the same concentrations. For example; 100mg/L of MPA recorded peak areas of 1.29, 19.3 and 38.1 arbitrary units [9]. Possible instrument error was eliminated by testing 1000mg/L standard solution 7 times which resulted in consistent peak areas [9]. The peaks areas for the instrument validation test was vastly different from the peak areas that was obtained from the 1000mg/L standard

(ranged between 176-406 while the validation test was at a consistent 500-530) which may actually suggest possible instrumental error at that particular time.

Despite the complications, Chua was able to create a calibration curve which would measure the concentration of underivatised MPA in the aqueous layer in order to assess the efficiency of the two-phase derivatisation. From a 1000mg/L sample of MPA, it was calculated that only 14.6% (approx. 146mg/L) of derivatised MPA was found in the organic layer [9]. When Chua attempted to analyse the derivatives again 210 minutes later, all 3 samples recorded lower peak areas than the initial test [9]. The three control samples each containing 1000mgl/L were then analysed 60 minutes after and all recorded significantly lower peak areas than the previous runs [9]. The peak areas of the controls should theoretically be higher than the derivatized samples as it contains the full 1000mg/L of MPA without derivatisation. The study shows that although methylphosphonic acid can be derivatised and analysed using gas-chromatography using this two-phase derivatisation method, only 14.6% of MPA was derivatised into the organic layer. This value however, can be questioned due to inconsistencies with the analysed peak area from the calibration standards, controls and derived samples.

It is difficult to determine the cause of inconsistent peak areas. Possible equipment error as mentioned could explain the fluctuating results from the calibration curve. Results also suggest possible sample degradation due to the declining peak areas. MPA in an aqueous state is a very stable compound. The main reason methylphosphonic acid is used as a marker for organophosphorus nerve agents was due to it's persistence in the environment [18,23,24]. Mills has reported that the half-life of MPA in water is estimated to be approximately 18 years [36]. Mill also mentions that MPA is also very resistant to light thus removing the

possibility of UV degradation [36]. Chua suggested that the cause for the discrepancies was due to MPA adsorbing onto laboratory equipment.

5.0 ADSORPTION ONTO LABORATORY GLASSWARE

Adsorption is the phenomenon in which a gas or liquid adheres and builds up on the surface of a solid [10]. Adsorption onto laboratory equipment is quite a common issue in all fields of science. In analyses dealing with trace concentrations, loss of concentration due to adsorption on laboratory equipment can be detrimental to the reliability of the results [12,13]. Untreated glass equipment contains silanol groups on the surface which are hydrophilic in nature [12,13]. Chemical adsorption onto glassware occurs due to ionic exchange at these silanol sites [39].

Ackerman states that non-polar molecules in aqueous solutions have a strong affinity for glass and Teflon [40]. Ackerman's study determined if polycyclic aromatic hydrocarbons adsorbed onto laboratory glassware. Although the study was to improve solid-phase micro extractions using 1PS paper, concentrations of solutes were recorded to be lost due to adsorption on both glass vials and stirrer bars [40]. Ackerman also used polar solutes as a comparison and observed that even polar compounds can adsorb onto these particular surfaces [40]. Fenimore also supports this stating that irreversible adsorption is of polar compounds at the microgram and sub-microgram levels is a frequent issue [50]. Methylphosphonic acid being polar can exert these characteristics and it wouldn't be impossible to assume that MPA can readily adsorb onto glassware.

In many studies regarding adsorption to glassware, studies prior often ignore this factor although mentioning the possibility of it occurring [10,13,42]. Eichholz in 1965, strengthens this statement in his study on radioactive isotopes and the effects of glassware adsorption where it was stated that total adsorption on glassware is so small that it can be neglected in the majority of radiochemical and trace analysis. Eichholz further comments that coating

glassware in a hydrophobic agent does reduce adsorption, but is not worth the trouble and expenses for everyday analyses [13].

According to Roth, there are two different types of adsorption that contribute to the loss of concentration. Adsorption due to equilibrium conditions suggests the loss of concentration on a surface over a particular amount of time [59]. A study on THC-COOH reports that concentrations can decrease from up to 46% over a 5-hour period, although the concentration loss was less than 10ng/cm^2 [59]. Adsorption due to kinetic conditions suggests the loss of concentration that occurs when the sample comes into contact with a surface and is then removed e.g. pipetting [59]. In the same study, 8% to 57% of the original concentration can be lost due to rapidly pipetting the same solution. Losses however were far smaller than that recorded from equilibrium conditions [59].

5.1 Proteins and Peptides

Adsorption of proteins is a major concern in biology, medicine and food processing [41]. There are many possible factors that contribute to proteins adsorbing to surfaces. Proteins and peptides are amphipathic which means they possess both a polar and non-polar end which makes them readily adsorb onto most surfaces [37,38,42]. Because of this, Nakanishi states that the interaction between proteins and surfaces becomes complicated, and hydrophilic and hydrophobic forces become hard to predict [41]. Ionic amine-silanol bonding and hydrogen bonding are the main driving forces for adsorption of proteins to glass surfaces according to Messing. The rate of adsorption depends on the number of amine groups contained in the protein as well as the weight [43].

Karlsson suggests that the driving force behind protein adsorption was the stability of the protein [45]. Karlsson found that stable proteins are less prone to adsorb onto a solid surface,

protein with better stability will adsorb slower and increase in protein stability leads to an increase in its ability to desorb [45].

Despite this, there are situations in which protein adsorption is a desired effect. Adsorption of proteins is useful in areas such as the development of chromatography materials and production of combined and adsorbed vaccines [45].

External factors such as temperature, pH, ionic strength and buffer composition can also contribute to the adsorption behaviour of proteins [66].

Midwoud has stated that compared to proteins, studies on why peptides adsorb to glassware and plasticware is less studied and documented [38]. Maes suggests that peptides adsorb onto glassware due to electrostatic interactions between the positively charged peptides and the negatively charged silanol groups [56]. Maes also suggests that peptides adsorb to plastic due to hydrophobic reactions [56,59]. Kristensen study on cationic membrane-active peptides, states that when conducting experiments using typical peptide concentrations, up to 90% of the concentration can be lost due to rapid adsorption to the walls of the containers [44].

Both peptides and proteins adsorption effects have affected the reliability of GC-MS and LC-MS analysis. Poor repeatability of peak areas is a frequent issue in the analysis of these biochemical using LC-MS [38]. Adsorption of proteins and peptides can occur in potentially every component of the machine such as the column, tubing, sampler and even the mass spectrometer [56]. This only occurs when analysing hydrophobic compounds in which MPA is not due to its suitability with LC-MS.

5.2 Glass vs Plastic

Studies have also been conducted to determine if there was a difference in using plastic equipment instead of glassware equipment. Plastic equipment is not only cheaper than glass but is also less prone to breaking which increases safety [46]. Due to the nature of proteins and peptides, it becomes very difficult to recommend one particular type of container in order to optimally minimise adsorption [42]. Goebel-Stengal looked to determine which glassware and/ or plasticware should be used when handling and storing peptides. The study concluded that all 8 of the tested peptides reacted to each set of glassware and plasticware differently in which it was difficult to recommend one solution [42]. Even when analysing net charge, hydrophobicity, chain length and charge distribution, it was not enough to predict which container to use that would minimise adsorption and optimise peptide recovery [42].

Preissner studied the effects of hormones and proteins on glass and plastics to determine which material was more optimal over the course of seven days. The test found no significant difference between the two types of equipment and that any differences were small enough to be deemed clinically insignificant [46]. Preissner however, corroborates with Karlssons results of protein stability as the protein cancer antigen-125 (CA-125) which was the least stable of all the tested proteins, had decreased concentration in all storage containers over the seven-day testing period [45,46]. Suelter has suggested that adsorption of proteins can be minimized by modifying the solvent in which proteins are kept instead of modifying the container and or its surface [37]. Use of glycerol (50%) or Triton X-100 (0.2mM) as the solvent provided better protection from adsorption in both plastic and glass containers compared to coating the surfaces in bovine serum albumim [37].

It is unsure which type of container is best suited for containing peptides. Midwoud has had success in using glass as it was able to improve the repeatability of peptide analysis using

LC-MS [38]. This is contradicted in Vatansever's study where plastic vials had performed better than glass vials in order to improve peptide analysis using mass spectrometry [57].

Use of borosilicate glassware when handling radioactive isotopes is more preferable compared to plastic containers although cesium, ruthenium and zirconium are less contaminated in plastic [10]. Adsorption losses to these surfaces are small and can be overlooked. A study examining adsorption characteristics of silver, lead, cadmium, zinc and nickel found that neither glass or plastic prevented adsorption of all 5 metals to a satisfactory level [47]. Borosilicate glassware and acidification of the metal using nitric acid did perform better than plastic as it was able to minimise the concentration loss of silver, lead, cadmium and zinc [47]. Roth has also shown that containing chemical THC-COOH in untreated glass, provides the least amount of loss due to adsorption, comparing it to plastic containers made from polyethylene and polypropylene [59]. Roth also mentions that loss of concentration due to adsorption can also be caused by the type of solvent and the amount of exposed surface area, not just the type of container [59].

5.3 Silylation of Glassware

A possible solution in order to minimise the adsorption that occurs on glass surfaces is by silanizing the glassware. Silylation of glassware involves reacting the glass surface with a silicon group in order to increase the hydrophobicity of the glass [11,50,67]. Chlorosilanes reacts with the silanol groups that are found on untreated glass surfaces to form a siloxane cap, which coats the glass surface in a hydrophobic layer, and hydrochloric acid (HCl) as a

by-product [11].

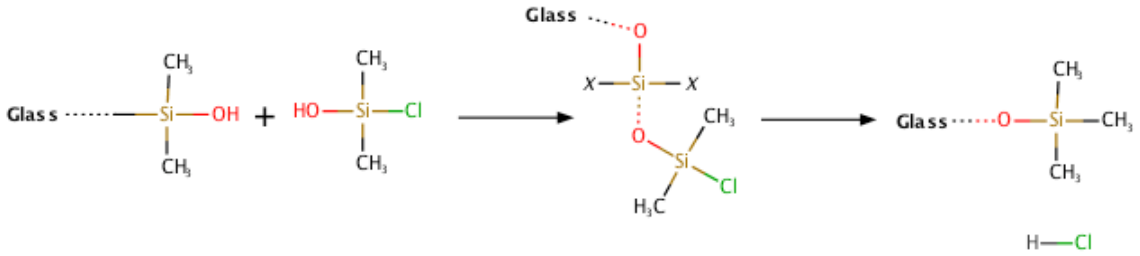


Figure 3: Silylation Reaction of Silanol and Trimethyllchlorosilane to form a Trimethylsiloxane Cap and HCl. Adapted and Modified from Seed B. [11]

5.3.1 Method for Silylation

As silanizing glassware is to coat the glass using siloxane, there are many different methods to achieve this result. The most common method is by treating glassware with dimethyldichlorosilane in toluene [11]. The glassware is then rinsed with methanol to convert any Si – Cl groups to Si – OCH_3 group [11]. Subramaniam has had success in submerging glass vials in dichloromethylsilane before baking them in the oven [48]. RNA Methodologies suggests using a pre–mixed reagent called Sigmacote to silylate glassware. Sigmacote is a silicon solution that also contains heptane that when applied to the required area, drained and then dried, is able to coat the glassware with siloxane. The drained solution can also be reused provided that no moisture was present on the glassware [49]. This method is used to prevent adsorption of RNA which is polar therefore there is a good possibility that this reagent may have success in minimising adsorption of MPA onto glassware.

Submerging glassware in solvents has some drawbacks especially on a commercial scale as disposal of large amounts of flammable and toxic solvent becomes a challenge. HCl can also be a product when using dichlorosilanes if water is present [50]. Fenimore in 1982, developed a method to silylate glassware using hexamethyldisilazane vapour and

polymerizing it to glassware in a vacuum oven [50]. The method produces glassware that performed on par to glassware that had been submerged in dichlorosilanes. Although not as simple compared to submerging glassware, it minimizes the issues that come with submerging [50]. Seed's method involves evaporating either dichlorodimethylsilane or trichlorodimethylsilane via vacuum and then sealing the silane vapours and glassware in a desiccator to allow the vapours to polymerise on the surface [11]. Armarego also uses a similar approach, instead opting to use dichloromethylsilane [67]. Armarego has even provided a method to silanize plasticware using the same method but instead of baking in the oven, treated plasticware should be thoroughly rinsed in with water [67]. This method is more practical for laboratories that don't have access to a vacuum oven but still runs the risk of using dichlorosilanes. Substituting the dichlorosilanes with hexamethyldisilazane as per Fenimore's method can be possible as both methods apply the same theory, but using different equipment.

Deyhimi suggests that when attempting to silylate glass made from sodium borosilicate, silane reagents with amino groups e.g. (dimethyl amino) trimethylsilane and bis (dimethyl amino) dimethylsilane should be used [51]. Deyhimi also suggests that silane reagents containing multiple functional groups produced more hydrophobicity compared to its mono-functional counterparts due to steric hindrance [51]. Of the 6 silanizing reagents tested, Deyhimi ranks the effectiveness of the reagents in providing hydrophobicity as:

Table 5: Six Silanizing Reagents Ranked in Effectiveness. Adapted from Deyhimi F. [51]

Rank	Reagent
1st	Bis(dimethylamino)dimethylsilane
2nd	Dimethyldichlorosilane

3rd	hexamethyldisilazane
4th	(dimethylamino)trimethylsilane
5th	Tributylchlorosilane
6th	trimethylchlorosilane

Glassware must also be cleaned prior to silanization to minimise any contamination that will decrease the effectiveness of the hydrophobic coating. Subramaniam used nitric acid and Fenimore used dilute HCl for their methods [48,50]. In a study to determine the most optimal cleaning method, Cras concluded that a 1:1 methanol to HCl wash followed by sulfuric acid was the best method in order to achieve an even silanization on glass surfaces [55].

5.3.2 Effectiveness of Silylation

Silylation of glassware has proven to be a very effective method in order to reduce irreversible adsorption to glassware in various different situations. Subramaniam in 2010, developed a method to rapidly screen and identify APAs that has been derivatised with fluorinated phenyldiazomethane for detection of organophosphorus nerve agents. Subramaniam opted to use silylated glassware in order to avoid irreversible adsorption of alkylphosphonic acid. In 19 aqueous samples, silylated glassware assisted in improving the yield of methylphosphonic acids by 20% [48] and commented that when dealing with trace concentrations of APAs (ng/ml), silylation is essential.

Silylation of glassware has prevented significant concentration loss in storing mercury with concentrations as low as 1ng/L [53]. Naykki suggests that this method could be used to handle other metals in trace amounts but states that good laboratory practices are more important to prevent loss of concentration [53].

Silylated glassware was proven to be more reliable in Ikeda's study when developing a quantification method for olanzapine in human plasma. Irreversible adsorption was an issue as the nitrogen atoms found on olanzapine would react to silanol groups that are located on the surface of glassware thus decreasing the known concentration of the sample [52]. Silylated glassware did not completely suppress surface activity, but was able to provide a fair larger recovery yield compared to the untreated glassware as all but 1 scenario had recovery yields no less than 90% [53].

Williams study in 2016 assessed the efficiency of five silane reagents to treat glass slides with depressions/ channels in order to minimise the adsorption of proteins on these channel walls. Testing each coating under different properties such as hydrophilicity, stability and durability, Williams concluded that coating glass using a zwitterionic sultone derived silane (ZS) was the most effective method to prevent surface activity of immunoglobulin and bovine serum alumin on glass. However, ZS coatings lose its effectiveness over time due to degradation. For experiment durations over 6 hours, Willams suggest the use of 2-[methoxy(polyethleneoxy)] propyl trimethoxysilane (MPEG) for better stability despite having less protection to surface activity [54].

Goebel-Stengal despite concluding that there was no best solution to optimise recovery of peptides, did have success by siliconizing glassware which can further be improved with the addition of bovine serum albumin in order to improve recovery of peptides [42].

6.0 Conclusion

Current methods of detecting and analysing nerve agent degradants within the environment are often time consuming and ineffective. Degradants such as MPA need to be derivatized so that it becomes suitable for GC-MS. Derivatisation requires the elimination of water which is a major source of error due to its difficulty and time. MPA was successfully detected using GC-MS using the Dival's proposed method of derivatizing MPA with an addition of an organic layer with a LOD of 1000ppm [8]. A quantitative study using LC-MS was conducted by Chua to test the efficiency of this proposed method which was concluded that only 14% of the available MPA was able to be derivatized [9]. The reproducibility of this result is questioned due to the loss in concentration of MPA over time. Chua proposed that the loss of concentration was due to irreversible adsorption onto laboratory equipment. This phenomenon is often overlooked in some studies but is crucial in studies dealing with bio-chemicals such as proteins and peptides. Silylation or silconizing of glassware has proven to be a successful method in minimising the adsorption of MPA in Subramaniam's research however, no other studies involving MPA or nerve agent degradants have been reported. Silylation has had success in minimising adsorption onto glassware other fields. Evaluation of silylation and silconizing methods need to be considered in order to develop a method that best minimises adsorption. Considerations regarding the laboratory equipment has to be made in order to minimise this phenomenon in order to determine the reliability of the quantitative study and hopefully improve Chua's method in developing an analytical method for the analysis of MPA derivatives using LC-MS.

References

[1] Chauhan S, D'Cruz R, Faruqi S. Chemical Warfare Agents. Environmental Toxicology and Pharmacology. 2008;26(2):113-22.

[2] Hooijschuur E, Kientz C, Brinkman U. Analytical separation techniques for the determination of chemical warfare agents. Journal of Chromatograph A. 2002;982(2):177-200.

[3] Munro N, Talmage S. The Sources, Fate, and Toxicity of Chemical Warfare Agent Degradation Products. Environmental Health Perspectives. 1999;107(12):933-74.

[4] Otsuka M, Tsuge K, Seto Y. Analysis of Degradation products of nerve agents via post-pentafluorobenzylation liquid chromatography-tandem mass spectroscopy. Journal of Chromatography A. 2018;1577:31-7.

[5] Black RM. History and perspectives of bioanalytical methods for chemical warfare agent detection. Journal of Chromatography B. 2010;878(17-18):1207 - 15.

[6] Baygildiev T, Zatirakha A, Rodin I. Rapid IC-MS/MS determination of methylphosphonic acid in urine of rats exposed to organophosphorus nerve agents. Journal of Chromatography B. 2017;1058:32-9.

[7] Seto Y, Tachikawa M, Kanamori-Kataoka M. Target Analysis of tert-butyldimethylsilyl Derivatives of Nerve Agent Hydrolysis Products by Selectable One-Dimensional or Two-Dimensional Gas Chromatography–Mass spectrometry. Journal of Chromatography A. 2017;1501:99-106.

[8] Dival L. The Development of an in-field Rapid Derivatisation Technique for the analysis of Chemical Warfare Agents Degradants [dissertation]. 2018.

[9] Chua X. Derivatisation of Chemical Warfare Agent Degradant Without Removal of Water [Project]. 2018.

[10] Inglezakis V, Pouopoulos S. Adsorption, Ion Exchange and Catalysis. Design of

Operations and Environmental Applications. 2006:31-56.

[11] Seed B. Silanizing Glassware. Current Protocols in Cell Biology. 2001;8(1).

[12] Knapp D. Handbook of Analytical Derivatization Reactions: Wiley; 1979.

[13] Eichholz G, Nagel A, Hughes R. Adsorption of Ions Dilute Aqueous Solutions on Glass and Plastic Surfaces. Analytical Chemistry. 1965;37(7):863-8.

[14] Marrs T, Maynard R, Sidell F. Chemical Warfare Agents: Toxicology and Treatment 2nd ed: John Wiley & Sons 2007.

[15] Weissberg A, Madmon M. Determination of Organophosphorus Acids by Liquid Chromatography Positive Electrospray Ionization Tandem Mass Spectrometry after Chemical Derivatization. International Journal of Mass Spectrometry. 2016;408:20-7.

[16] Mercey G, Verdelet T, Renou J. Reactivators of acetylcholinesterase inhibited by organophosphorus nerve agents. Accounts of Chemical Research. 2012;45(5):756-66.

[17] Vucinic S, Antonijevic B, Tsatsakis A. Environmental Exposure to Organophosphorus Nerve Agents. Environmental Toxicology and Pharmacology. 2017;56:163-71

[18] Minami M, Hui D-M, Inagaki H. Method for the Analysis of the Methylphosphonic Acid Metabolites of Sarin and its Ethanol-Substituted Analogue in Urine as Applied to the Victims of the Tokyo Sarin Disaster. Journal of Chromatography B: Biomedical Sciences and Applications 1997;695(2):237-44.

[19] Vale JA, Marrs T, Maynard R. Novichok: a murderous nerve agent attack in the UK. Clinical Toxicology. 2018;56(11):1093-7.

[20] Jokanovic M. CHAPTER 52 - Metabolism of Warfare Nerve Agents: Academic Press; 2009. 799-810 p.

[21] Verweij A, Mensingh G, Boter H. The Occurrence of PCH3-containing compounds in surface waters Chemosphere. 1982;11(10):985-90.

[22] Mesilaakso M. Chemical Weapons Convention chemicals analysis: sample collection, preparation, and analytical methods. 1st ed. Hoboken: Wiley; 2005.

[23] Rohrbaugh D, Sarver E. Detection of alkyl methylphosphonic acids in complex matrices by gas chromatography-tandem mass spectroscopy. Journal of Chromatography A. 1998;809(1-2):141-50.

[24] Tripathi D, Pandey K, Bhattacharya A. Mass spectrometric identification of methyl phosphonic acid: the hydrolysis product of methyl phosphonofluoridate and pinacolyl methyl phosphonofluoridate Analytical Chemistry. 1992;64(72):823-34.

[25] Smith P. Chapter 27 - Chemical Warfare Agents: Elsevier; 2012. 621-46 p

[26] Nakajima T, Sasaki K, Ozawa H. Urinary metabolites of sarin in a patient of the Matsumoto sarin incident. Archives of Toxicology. 1998;72(9):601-3.

[27] D'Agostino P, Chenier C. Analysis of Chemical Warfare Agents: General Overview, LC-MS Review, In-House LC-ESI-MS Methods and Open Literature Bibliography. Canada: Defence Research and Development Canada; 2006.

[28] Read R, Black R. Rapid screening procedures for the hydrolysis products of chemical warfare agents using positive and negative ion liquid chromatography-mass spectrometry with atmospheric pressure chemical ionisation Journal of Chromatography A. 1999;862(2):169-77.

[29] Black R, Read R. Analysis of degradation products of organophosphorus chemical warfare agents and related compounds by liquid chromatography-mass spectrometry using electrospray and atmospheric pressure chemical ionisation. Journal of Chromatography A. 1998;794(1-2):233-44.

[30] Mawhinney D, Stanelle R, Hamelin E. Enhancing the Response of Alkyl Methylphosphonic Acids in Negative Electrospray Ionization Liquid Chromatography Tandem Mass Spectrometry by Post-Column Addition of Organic Solvents. Journal

of the American Society for Mass Spectrometry. 2007;18(10):1821-6.

[31] Black R, Muir B. Derivatisation reactions in the chromatographic analysis of chemical warfare agents and their degradation products. Journal of Chromatography A. 2003;1000(1-2):253-81.

[32] Creasy W, Rodriquez A, Stuff J. Atomic emission detection for the quantification of trimethylsilyl derivatives of chemical-warfare-agent related compounds in environmental samples. Journal of Chromatography A. 1995;709(2):333-44.

[33] Blau K, Halket J. Handbook of derivatives for chromatography: Wiley; 1993.

[34] Purdon J, Pagotto J, Miller R. Preparation, stability and quantitative analysis by gas chromatography and gas chromatography-electron impact mass spectrometry of tert-butyldimethylsilyl derivatives of some alkylphosphonic and alkyl methylphosphonic acids. Journal of Chromatography A. 1989;475(2):261-72.

[35] Enqvist J, Rautio M. Identification of degradation products of potential organophosphorus warfare agents. Helsinki: Ministry for Foreign Affairs of Finland. 1980.

[36] Mill T, Gould CW. Free-radical oxidation of organic phosphonic acid salts in water using hydrogen peroxide, oxygen, and ultraviolet light. Environmental Science & Technology. 1979;13(2):205-8.

[37] Suelter CH, DeLuca M. How to prevent losses of protein by adsorption to glass and plastic. Analytical Biochemistry. 1983;135(1):112-9.

[38] Midwoud P, Rieux L, Bischoff R. Improvement of Recovery and Repeatability in Liquid Chromatography-Mass Spectrometry Analysis of Peptides. Journal of Proteome Research. 2007;6(2):781-91.

[39] Asakawa N. The Horror of Sample Adsorption to Containers: SHIMADZU: Excellence in Science

[40] Ackerman A, Hurtubise R. The effects of adsorption of solutes on glassware and Teflon in the calculation of partition coefficients for solid-phase microextraction with 1PS paper. Talanta. 2000;52:853-61.

[41] Nakanishi K, Sakiyama T, Imamura K. On the adsorption of proteins on solid surfaces, a common but very complicated phenomenon. Journal of Bioscience and Bioengineering. 2001;91(31):233-44.

[42] Goebel-Stengal M, Stengal A, Tache Y. The importance of using the optimal plasticware and glassware in studies involving peptides. Analytical Biochemistry. 2011;414(1):38-46.

[43] Messing R. Adsorption of proteins on glass surfaces and pertinent parameters for the immobilization of enzymes in the pores of inorganic carriers. Journal of Non-Crystalline Solids. 1975;19:277-83.

[44] Kristensen K, Henriksen JR, Andresen TL (2015) Adsorption of Cationic Peptides to Solid Surfaces of Glass and Plastic. PLoS ONE 10(5)

[45] Karlsson M, Ekeroth J, Elwing H. Reduction of Irreversible Protein Adsorption on Solid Surfaces by Protein Engineering for Increased Stability. The Journal of Biological Chemistry 2005;280(27):25558-64.

[46] Preissner C, Reilly W, Cyr R. Plastic versus Glass Tubes: Effects on Analytical Performance of Selected Serum and Plasma Hormone Assays. Clinical Chemistry. 2004;50(7):1245-7.

[47] Struempler A. Adsorption characteristics of silver, lead, cadmium, zinc, and nickel on borosilicate glass, polyethylene, and polypropylene container surfaces. Analytical Chemistry. 1973;45(13):2251-4.

[48] Subramaniam R, Astot C, Juhlin L. Direct Derivatization and Rapid GC-MS Screening of Nerve Agent Markers in Aqueous Samples. Analytical Chemistry.

2010;82(17):7452-9.

[49] APPENDIX H - Silanizing Centrifuge Tubes and Glassware RNA Methodologies (Third Edition). 2005:676.

[50] Fenimore DC, Davis CM, Whitford JH. Vapor Phase Silylation of Laboratory Glassware. Analytical Chemistry. 1976;48(14):2289-90.

[51] Deyhimi F, Coles J. Rapid Silylation of a Glass Surface: Choice of Reagent and Effect of Experimental Parameters on Hydrophobicity. Helvetica Chimica Acta. 1982;65(6):1752-9

[52] Ikeda K, Ikawa K, Kozumi T. Development and Validation of a GC-EI-MS method with reduced adsorption loss for then quantification of olanzapine in human plasma. Analytical and Bioanalytical Chemistry. 2012;403(7):1823-30.

[53] Naykki T. The Use of silylation for minimizing the interference effects caused by contamination in ultra-low level mercury analytics. Analytical and Bioanalytical Chemistry. 2002;372(7-8):829-31.

[54] Williams S, Venkateswaran N, O'Donnell T. Assessing Stability, Durability, and Protein Adsorption Behaviour of Hydrophillic Silane Coatings in Glass Microchannels. Journal of Analytical and Bioanalytical Techniques. 2016;7(3):318.

[55] Cras JJ, Rowe-Taitt C, Nivens D. Comparing of chemical cleaning methods of glass in preparation for silanization. Biosensors and Bioelectronics. 1999;14(8-9):683-8.

[56] Maes K, Smolders I, Michotte Y. Strategies to reduce aspecific adsorption of peptides and proteins in liquid chromatography-mass spectrometry based bioanalyses: An overview. Journal of Chromatography A. 2014; 1358:1-13.

[57] Vatansever B. Comparison between linear ion trap and a triple quadruple MS in the sensitive detection of large peptides at femtomole amounts on column. Journal of Separation Science. 2010;33(16):2478-88.

[58] Baygildiev TM, Robin IA, Stavrianidi AN. Time-Efficient LC/MS/MS Determination of Low Concentrations of Methylphosphonic Acid. Inorganic Materials. 2017;53(14):1382-5.

[59] Roth K. Investigation of the Effects of Solution Composition and Container Material Type on the Loss of 11-nor-Δ^9 -THC-9-Carboxylic Acid. Journal of Analytical Toxicology. 1996;20:291-300.

[60] Kataoka M, Tsuge K, Takesako H. Effect of Pedological Characteristics on Aqueous Soil Extraction Recovery and tert-Butyldimethylsilylation Yield for Gas Chromatography-Mass Spectrometry of Nerve Gas Hydrolysis Products from Soils. Environmental Science and Technology. 2001;35(9):1823-9.

[61] Rautio M. Recommended operating procedures for sampling and analysis in the verification of chemical disarmament. Ministry of Foreign Affairs of Finland. Helsinki; 1994.

[62] Riches J, Morton I, Read RW. The trace analysis of alkyl alkylphosphonic acids in urine using gas chromatography–ion trap negative ion tandem mass spectrometry. Journal of Chromatography B. 2005;816(1):251-8.

[63] Kataoka M, Seto Y. Discriminative determination of alkyl methylphosphonates and methylphosphonate in blood plasma and urine by gas chromatography–mass spectrometry after tert.-butyldimethylsilylation. Journal of Chromatography B. 2003;795(1):123-32.

[64] Weissberg A, Madmon M. Aqueous extraction followed by derivatization and liquid chromatography-mass spectrometry analysis: A unique strategy for trace detection and identification of G-nerve agents in environmental matrices. Journal of Chromatography A. 2018;1577:24-30.

[65] Weissberg A, Madmon M. Determination of trace amounts of G-type nerve agents in

aqueous samples utilizing “in vial” instantaneous derivatization and liquid chromatography-tandem mass spectroscopy. Journal of Chromatography A. 2017;1512:71-7.

[66] Rabe M, Verdes D, Seegar S. Understanding protein adsorption phenomena at solid surfaces. Advances in Colloid and Interface Science. 2011;162(1-2):87-106.

[67] Armarego W, Chai C. Purification of Laboratory Chemicals Butterworth-Heinemann 2009.

[68] Riches J. Chapter 7 - Analysis of Organophosphorus Chemicals. In: Timperley CM, editor. Best Synthetic Methods. Oxford: Academic Press; 2015. p. 721-52.

Part Two

Literature Review

DERIVATISATION OF NERVE AGENT DEGRADANTS WITHOUT REMOVAL OF WATER

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LIST OF ABBREVIATIONS

CWC	Chemical Weapons Convention
OPCW	Organisation for Prohibition of Chemical Weapons
APA	Alkylphosphonic Acid
MPA	Methylphosphonic Acid
GC–MS	Gas Chromatography–Mass Spectroscopy
MTBSTFA	*N*-methyl-*N*-*tert*-butyldimethylsilyltrifluoroacetamide
LC-MS	Liquid Chromatography – Mass Spectroscopy
MRM	Multiple Reaction Monitoring
ESI	Electrospray Ionisation
TIC	Total Ion Chromatogram
RSD	Relative Standard Deviation
PIS	Parental Ion Scan

ABSTRACT

A recent pilot study showed that methylphosphonic acid could be derivatised without the removal of water with the addition of an organic solvent into the reaction. A quantitative assessment was conducted to assess the efficiency of this method and to further optimize the previous quantitative study. A quick LC-MS analysis method for quantitative analysis of aqueous MPA was developed and optimized to give reproducible results with good peak shape and resolution. The limits of detection and quantification of this method were 0.134 ppm and 0.408 ppm respectively. MPA derivatised with MTBSTFA was able to be detected in the organic layer using GC-MS however the quantitative assessment of remaining MPA in the aqueous layer was inconclusive. Calculated peak areas suggest an increase in MPA concentration, which was due to poor sample preparation and evaporation of the aqueous layer. Addressing these issues in future studies will determine the effectiveness of this two phase derivatisation method.

Keywords: Methylphosphonic Acid, Liquid Chromatography – Mass Spectroscopy, Chemical Warfare Agents, Derivatisation Without Removal of Water

INTRODUCTION

The development, production, stockpiling and use of chemical warfare agents has been prohibited since the Chemical Weapons Convention (CWC) in 1997. About 130 countries have signed this treaty to disarm any of their stockpiled weapons to ensure that the chemicals are used for purposes that are not prohibited under the convention [1-3]. The Organisation for the Prohibition of Chemical Weapons (OPCW) conducts investigations for countries to ensure that the treaty is being held. Despite these measures, chemical warfare agents are still being utilised in terrorist attacks and wars even to this day [4].

Chemical warfare agents made from organophosphorus compounds are the deadliest form of chemical weapons. Examples of organophosphorus agents or nerve agents include sarin, tabun, soman, and VX. These chemicals are quite volatile however and in the presence of an aqueous environment, rapidly hydrolyse to form alkyl methylphosphonic acids (APAs) [1-3,5,6]. These products can undergo further hydrolysis to form the more stable methylphosphonic acid (MPA) as shown in figure 1 [1-3,5,6]

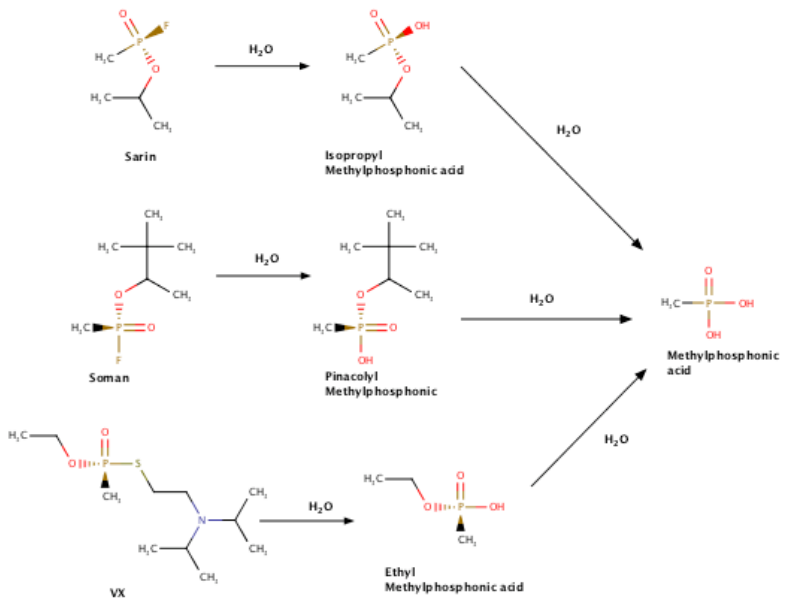


Figure 1: Hydrolysis Pathways for sarin, soman ,and VX to form MPA. Adapted and modified from Munro N. and Seto Y. [5,6]

MPA in water has an expected half life of 18 years and is very resistant to other forms of chemical degradation such as UV [7]. MPA is also not a naturally occurring chemical in the environment with very little uses, thus detection of MPA in the environment serves as a marker for the use of nerve agents [2,8].

The most popular technique used to analyse and identify chemical warfare agents and their degradants is via gas chromatography – mass spectroscopy (GC-MS) for it's unambiguous identification of compounds [3,9]. MPA is not suitable for this method due to its low volatility and polarity [10,11]. This issue can be overcome by derivatisation. A derivatisation reaction modifies a compounds functional groups to allow it to be more suitable for GC-MS [2,11]. Derivatisation however requires the evaporation of water from the aqueous samples to complete dryness, which has proven to be time consuming and can contribute to analytical errors [2,12-15].

In 2018, Dival developed a method that derivatised MPA for GC-MS analysis without the requirement of removing water. Dival suggested the addition of an organic solvent into the reaction. This creates a two phase solution, which would allow the MPA derivative to be detected in the organic layer. Dival successfully detected MPA that was derivatised using *N*-methyl-*N*-*tert*-butyldimethylsilyltrifluoroacetamide (MTBSTFA) and hexane, which was used as the organic solvent [16].

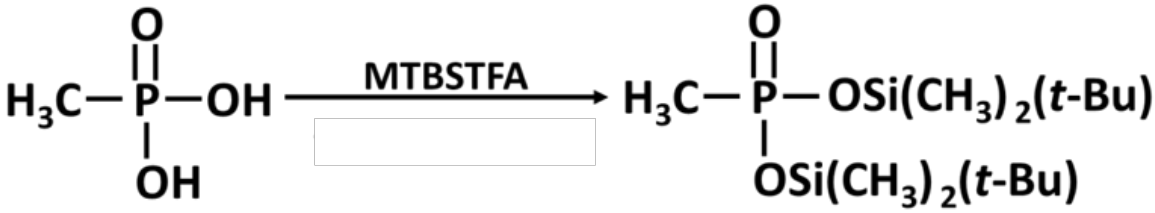


Figure 2: Derivatisation Reaction of MPA using MTBSTFA

A subsquent assessment regarding the efficiency of this method was conducted by Chua in 2018. The quantitative study aimed to determine the amount of unreacted MPA in the aqueous layer using liquid chromatography – mass spectroscopy (LC-MS). Chua concluded that the efficiency of the method to be 14.6 % at 1000 mg/L of MPA [17]. The calculated limit of detection and quantification of MPA in water were 1.6 mg/L and 5.3 mg/L respectively via LC-MS [17]. Reproducibility of the results in this study however could be questioned. Chua mentions that the inconsistent peak areas when constructing the calibration curve as well as the reduced peak areas of MPA over time, severely affected these results. A possible explanation could be irreversible adsorption of MPA onto the glassware.

This study aims to reassess Chua's study in order to perform a quantitative study on the two phase derivatisation method developed by Dival.

EXPERIMENTAL

Reagents and solvents:

Solid MPA and the derivatising reagent MTBSTFA were obtained from Sigma-Aldrich (St. Louis, MO, USA). Hexane was obtained from Thermo Fisher (Waltham, MA, USA). LC-MS grade acetonitrile and water for the mobile phases were obtained by Murdoch University from Merck (Victoria, AU). Formic acid in water (0.1%) was used as supplied at Murdoch University.

Standards and sample preparation:

A 1000ppm MPA stock solution was made up by dissolving 0.1064 g of solid MPA in 100 mL of deionised water (Accurate concentration: 1064 ppm). The stock solution was then serially diluted to create standards with concentrations of 200 ppm, 150 ppm, 100 ppm, 75 ppm, 50 ppm and 25 ppm in order develop the calibration curve.

Using the 100 ppm standard, another set of serial dilutions were conducted to create another set of standards with concentrations of 1000 ppb, 500 ppb, 250 ppb and 125 ppb to assist in determining the limit of detection for this LC-MS method.

Two-Phase Derivatisation

Another stock MPA solution was made by dissolving 0.1084 g of solid MPA in 100 mL of de-ionised water (Accurate concentration: 1084 ppm). Three 1 mL, 200 ppm solutions were then made from this stock solution and de-ionised water. To each of the MPA solutions, hexane (2 ml) and MTBSTFA (200 uL) were added and mixed vigorously in an oil bath at 60 °C for 30 minutes. The samples were then removed from the oil bath and left at room temperature for 15 minutes to allow the two phases to separate. The organic layer was then pipetted from each mixture and transferred into glass GC vials for analysis via GC-MS. The aqueous solution was then transferred into glass LC vials for analysis via LC-MS. Shimadzu MS certified vials were used for the LC-MS analysis.

Analytical Instruments

Analysis via LC-MS was conducted using the Shimadzu LCMS-8045 (Shimadzu Australiasia, Rydalmere, NSW, Australia) LC/MS/MS system equipped with a triple quadruple mass spectrometer. Separation of the analyte was conducted on the Nexera X2 liquid chromatography system fitted with a 2.0 mm x 50 mm Shim-pack XR-ODS III C18 column. Analysis was conducted using a multiple reaction monitoring (MRM) scan in negative electrospray ionisation (ESI) mode. Formic acid in water (0.1 %) (A) and acetonitrile (B) were used as the mobile phases. The mobile phases had a delivery flow rate of 0.3 mL/min with an elution gradient of 5 % B (0-2 min), ramped up to 90 % (2-2.5 min), maintained at 90% B (2.5-3.5 min), dropped back down to 5 % and then held for the

remainder of the time (3.5-4.5 min). Collision energy for the mass spectrometer was 15 V and oven temperature was 40 °C.

Analysis using GC-MS was performed using the Shimadzu GCMS-QP2010S (Shimadzu Australiasia, Rydalmere, NSW, Australia) coupled with a single quadrupole mass spectrometer with electron impact ionisation source. The machine was fitted with a BPX-5 (5 % phenyl polysilphenylene-siloxane) capillary column (30 m x 0.25 mm x 0.25 um). Splitless injections were used with the injection temperature maintained at 270 °C. Column temperature started at 80 °C and held for the first minute and then ramped up to 280 °C at a rate of 20 °C/min, which was then held for 6 minutes. The carrier gas used was ultra-high purity helium (BOC, Sydney, NSW, Australia) at 30 cm/sec. Analysis was performed in TIC (total ion chromatogram) mode with a scan rate of 555 scans/sec over the range of m/z 45 – 330.

Chromatograms and mass spectrums were visualized using Shimadzu Labsolutions program for both LC-MS and GC-MS.

Construction of calibration curves and data analysis

The calibration curve was developed using the 200 ppm, 150 ppm, 100 ppm, 75 ppm, 50 ppm, 25 ppm standards. Analysis via LC-MS was conducted with two blanks (one at the beginning and one at the end) and calibration standards in triplicates, approximately 30minutes apart in order to assess accuracy and linearity of the results. The entire analytical run was then re-conducted 24 hours later to assess reproducibility of the initial analytical runs results.

Another calibration curve was also developed using the 1000 ppb, 500 ppb, 250 ppb and 125 ppb standards to determine if it was possible to develop a calibration curve using this concentration range. Analysis via LC-MS using these standards was only conducted once. Limits of detection (LOD) and limits of quantification (LOQ) were calculated for each calibration curve. Relative standard deviation (RSD) was calculated for each calibration standard and sample in order to determine the precision and repeatability of the results. This was done using the following formula:

$$\%\text{RSD} = \frac{Standard\ Deviation\ (of\ the\ tripliciate)}{Average\ (of\ the\ tripliciate)} \text{ x } 100\%$$

Construction of the calibration curves was created using Microsoft Excel, plotting the peak area on the Y-axis vs concentration (ppm). A linear curve was generated from the plots.

RESULTS AND DISCUSSION

LC-MS method development:

Analysis using the Shimadzu LC-MS 8045 was conducted using an electrospray ionisation source in negative ion mode. De-protonating MPA would be the easiest method of ionising the compound due to it being an acid. Success however has been found with using both modes in previous studies involving this analytical method [2,14,17,18]. Both modes were directly compared using a 125 ppm MPA solution by a total ion count scan (TIC). Mass spectra for positive ionisation reveals a peak at m/z 97 which corresponds to MPA being protonated, $[M + H]^+$ as shown in figure 4. The mass spectrum for the negative mode shows a strong peak at m/z 95 which corresponds to MPA that has been deprotonated $[M – H]^-$ as seen in figure 5. A m/z 79 peak can also be seen on this mass spectra which corresponds to a fragmented MPA without a hydroxyl group.

Negative ionisation performed better when looking at the chromatograms. Both modes showed peaks at approximately 0.65-0.7 minutes which corresponded to MPA however, the negative ion mode chromatogram was more defined compared to the positive despite having better sensitivity as seen in figure 3. The positive ion mode chromatogram also contained other peaks which corresponded to the mobile phase. Using the 125 ppm sample, the detector was "overloaded" when using positive mode, which caused the undesirable peak shapes. An observation is that positive ionisation mode may be better suited for lower concentrations of MPA due to its better sensitivity.

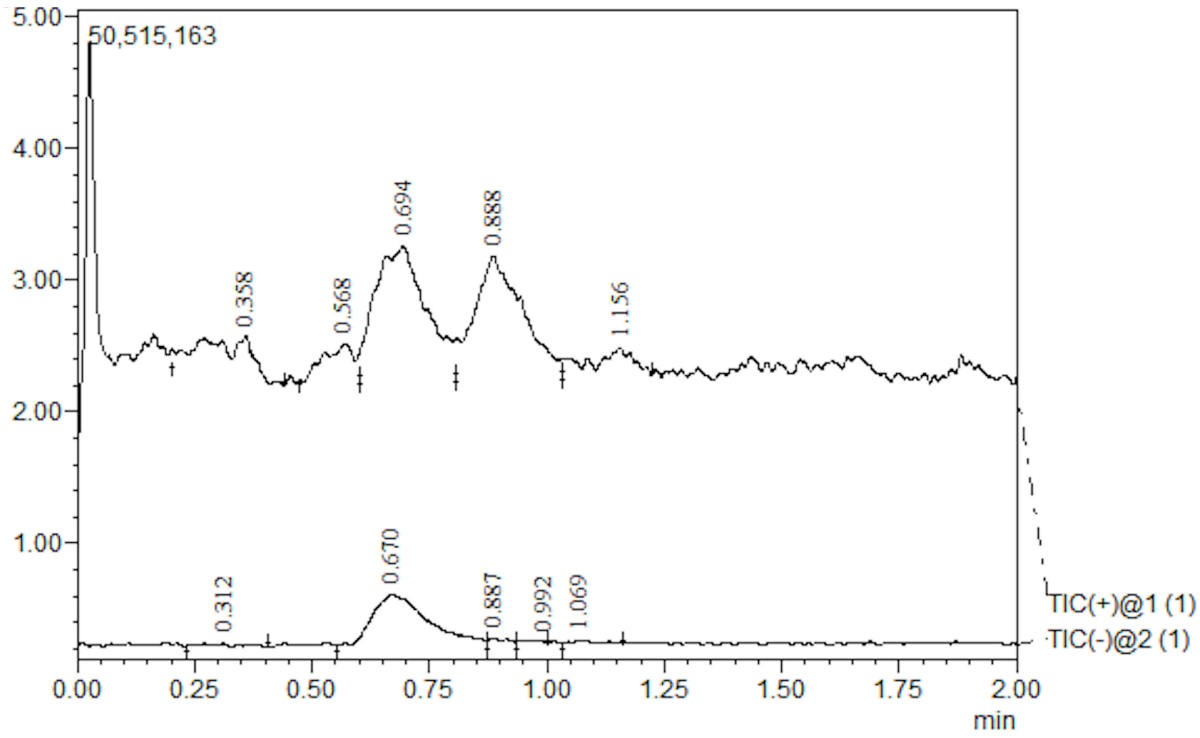


Figure 3: LC-MS Chromatogram for both positive and negative ESI modes. Analysed from a 125ppm MPA solution.

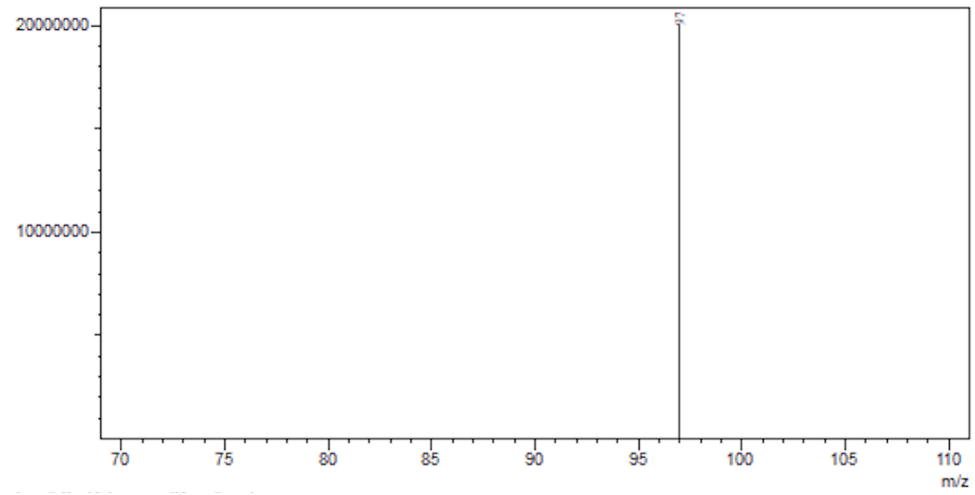


Figure 4: Mass Spectrum of MPA in positive mode from Figure 3. m/z 97 peak represents the protonated MPA

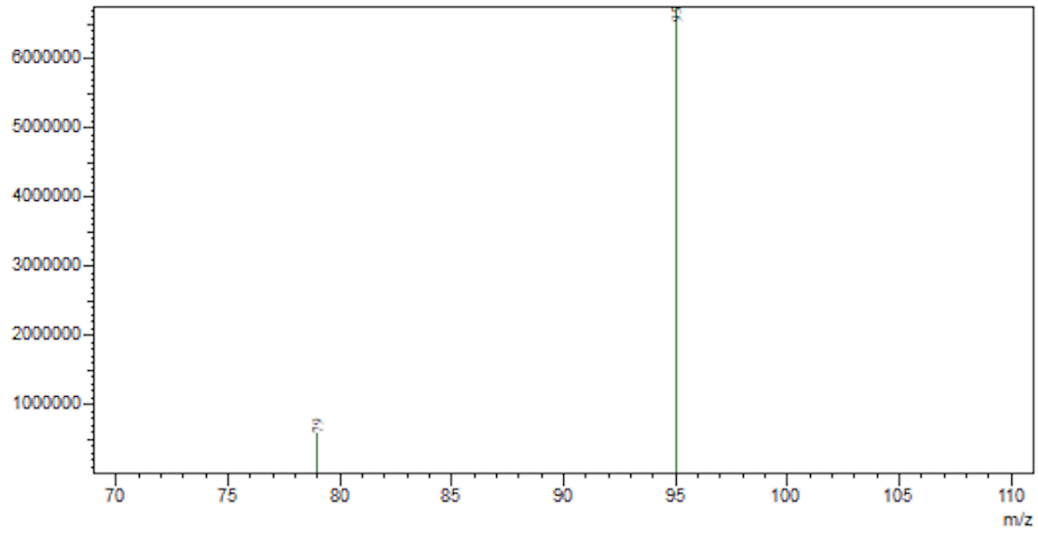


Figure 5: Mass spectrum of MPA in negative mode from Figure 3. m/z 95 represents the de-protonated MPA while m/z 79 is the fragmented MPA without a hydroxyl group

Despite this, the mass spectrometer was overloaded when injecting 1 μL of the 500 ppm standard in negative mode. A decision was made to scale the calibration curve to 25 ppm, 50 ppm, 75 ppm, 100 ppm, 150 ppm and 200 ppm rather than decreasing the injection volume due to concerns that there would be too little MPA being injected when dealing with the lower concentrations.

Water containing formic acid and acetonitrile were chosen as the mobile phases using Chua and Baygildiev's methods as an initial guideline [17,19]. Elution of MPA occurs at approximately 0.65 minutes using these mobile phases which already makes analysis times relatively short. Methanol as the organic solvent was also tested as there was a concern that the elution time of MPA was too quick. Using methanol did shift the chromatogram peak to the right by 0.05 minutes however, the intensity of the peak was less than the peak that was generated using acetonitrile. With both peak shapes being quite similar, we opted to use acetonitrile for better sensitivity.

15V was chosen as the collision energy because it gave rise to the most intense m/z 95 peaks during the parental ion scan (PIS), scanning for the m/z 95 peak.

The method could be conducted in an isocratic elution mode in order to minimize analysis time similar to Baygildiev [19]. The decision to conduct the method with a gradient elution was to ensure that any remaining contaminants in the system would be flushed out before the next injection. Figure 6 shows the chromatogram of the final LC-MS method.

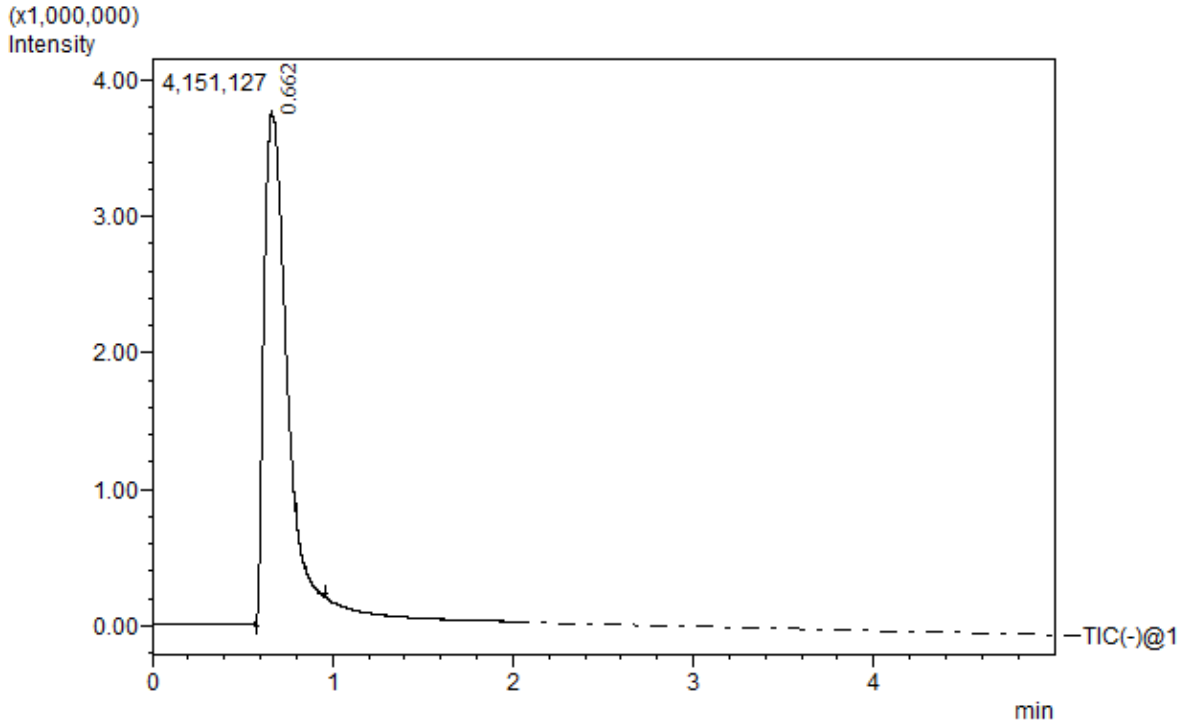


Figure 6: Liquid Chromatogram of the 150 ppm MPA calibration standard using the final optimized method

MPA Calibration curve:

The initial generation of the calibration curve was moderately successful. The 100 ppm standard gave peak areas that were lower than the 75 ppm standard, which suggests poor sample preparation for that particular standard. The 100 ppm standard was omitted when constructing the calibration curve. Despite this, reproducibility was very high with %RSD values of less than 1 % across all standards besides the 25 and 50ppm (5 and 8 % respectively) which is an improvement over Chua's study [17]. The LOD and LOQ were 19.94 ppm and 60.45 ppm respectively.

Table 1: MPA calibration standards: LC-MS peak areas, averages % %RSD

	Concentration (ppm)					
	25	**50**	**75**	**100**	**150**	**200**
Peak Area From Each	3163449	4609583	7409674	6463522	13755189	17270060
	2887499	4141256	7438260	6398165	13550944	17253915
	3146128	3989837	7386950	6366692	13658775	17130023

Triplicate Run						
Average	3065692	4246892	7411628	6409459.667	13654969.33	17217999.33
% RSD	5.04	7.61	0.35	0.77	0.75	0.44

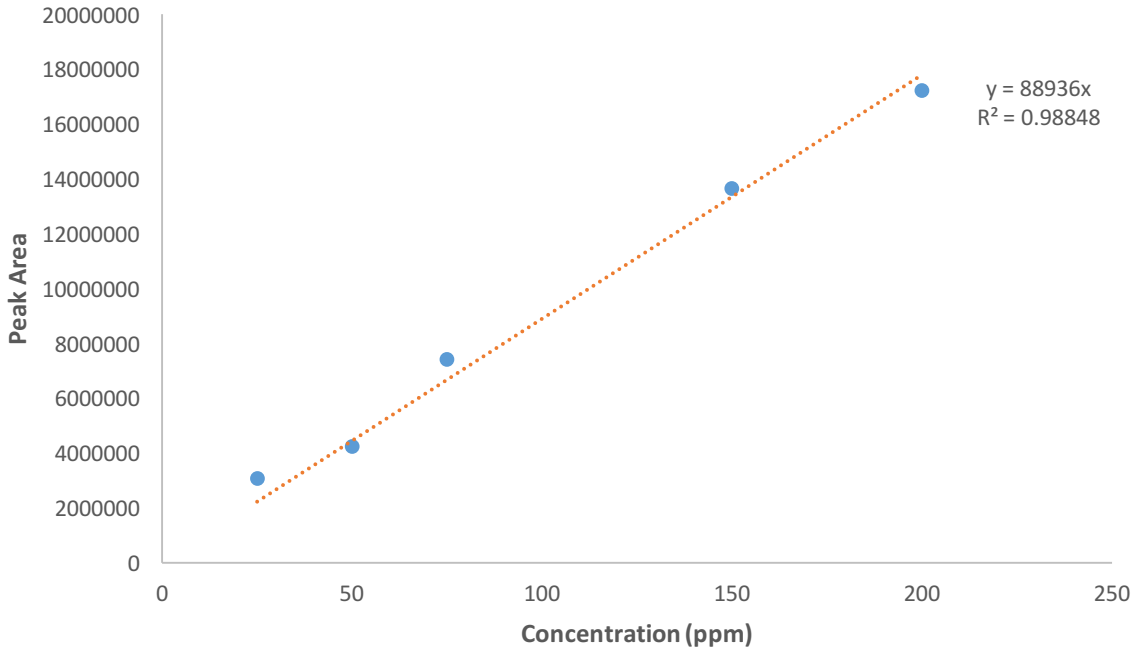


Figure 7: Calibration curve of MPA 25 ppm – 200 ppm

Table 2: Performance characteristics of the initial quantitative assessment of MPA

Analytical Range (ppm)	Equation	R^2	LOD (ppm)	LOQ (ppm)
25-200	Y = 88936x	0.98848	19.94	60.45

The analytical run was then re-conducted 24 hours later to further study the reproducibility of the results. A new 100 ppm standard was made to see if the linearity of the curve could be improved. Calculated peak areas were very similar to those that were generated 24 hours previously. Reproducibility was also very high as %RSD values were all close to 1 % besides the 25 ppm standard which was 8 %. Fluctuating peak areas in Chua's study could be

possibly due to instrument error and/or an un-optimized method. The calculated LOD and LOQ for this analytical run was 25.49 ppm and 77.25 ppm respectively which was higher than the previous day's calculated values.

Table 3: MPA calibration standards 24 hours later: LC-MS peak areas, average & %RSD

	Concentration (ppm)					
	25	**50**	**75**	**100**	**150**	**200**
Peak Area From Each Triplicate Run	2843614	4147282	7754493	10257335	14210127	17534747
	3254779	4298705	7680019	10050422	14106908	17424108
	2818821	4196832	7578644	10194521	13938005	17452508
Average	3049196.5	4214273	7671052	10167426	14158517.5	17470454.33
% RSD	8.03	1.83	1.11	1.04	0.97	0.33

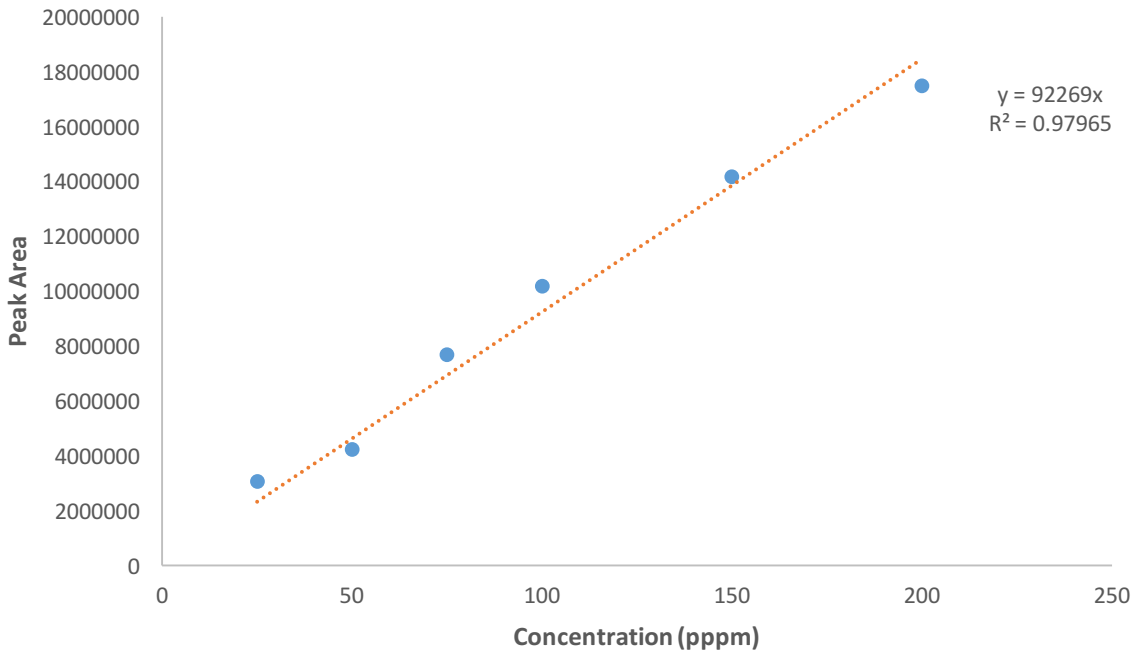


Figure 8: Calibration curve of MPA 25 ppm – 200ppm 24 hours later

Table 4: Performance characteristics of quantitative results 24 hours later

Analytical Range (ppm)	Equation	R^2	LOD (ppm)	LOQ (ppm)
25-200	Y = 92269x	0.97965	25.49	77.25

Another analytical run was conducted using a set of standards with a lower concentration range to see if it was possible to reduce the LOD and LOQ of this method. The LOD and LOQ of this run were 134.69 ppb (0.134 ppm) and 408.16 ppb (0.408 ppm). Bayglidiev had managed to achieve a limit of detection of 10 ppb (0.01 ppm) using a similar method, thus further optimization of analytical method could be explored [19].

Table 5: Quantitative results using a concentration range of 125-1000 ppb

Concentration (ppb)	Peak Area
125	3489
250	27430
500	51199
1000	116362

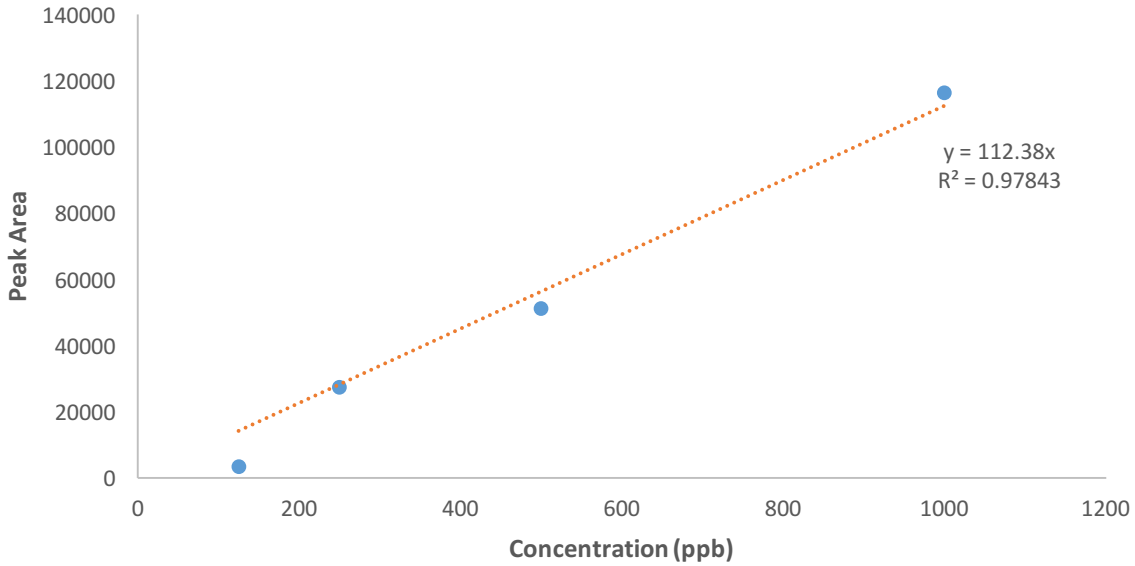


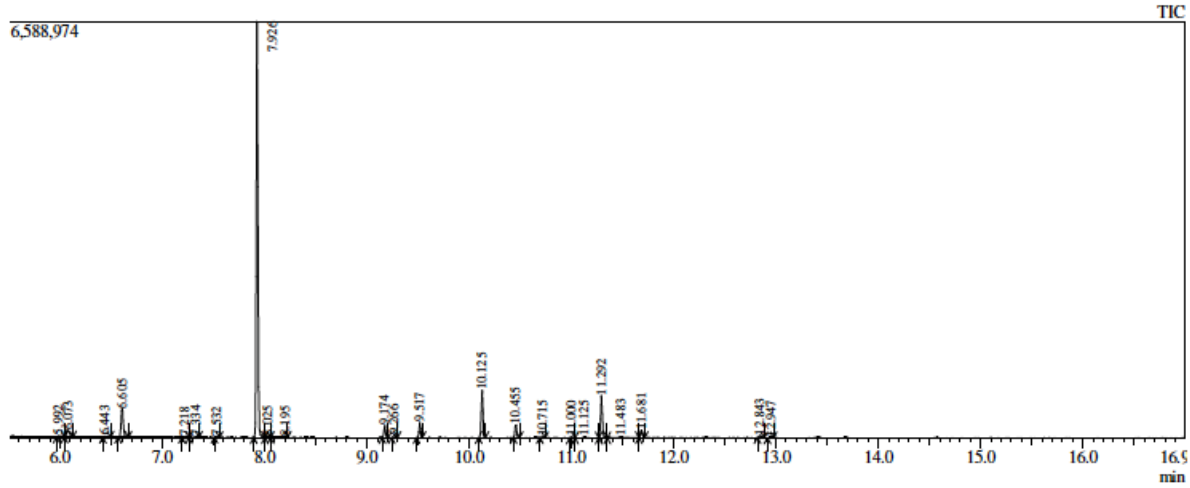
Figure 5: Calibration curve of quantitative results using a concentration range of 125-1000 ppb

Table 6: Performance characteristics of the quantitative assessment using a concentration range of 125-1000 ppb

Analytical Range (ppb)	Equation	R^2	LOD (ppb)	LOQ (ppb)
125-1000	Y = 112.38x	0.97843	134.69	408.16

Detection of MPA-derivative by GC-MS

Derivatisation of MPA using pure MTBSTFA was conducted following procedures that Dival and Chua had developed [16,17]. Because the calibration curves were developed with the maximum concentration being 200 ppm, only a 200ppm sample of MPA would be used for derivatisation despite Dival's study stating that the derivative could only be detected using 1000 ppm aqueous MPA [16]. The derivative was successfully detected in the organic layer using GC-MS. A large peak with retention time of 7.9 minutes was observed on the chromatograms for all three samples. The mass spectrum at this retention time contains a base peak at m/z 267 with a smaller m/z 73 peak. Similarity search on the Shimadzu database using the NIST library reveals that mass spectrum has a 94 % similarity to the mass spectrum for the MPA derivative bis[(dimethyl)(*tert*-butyl)siyl].



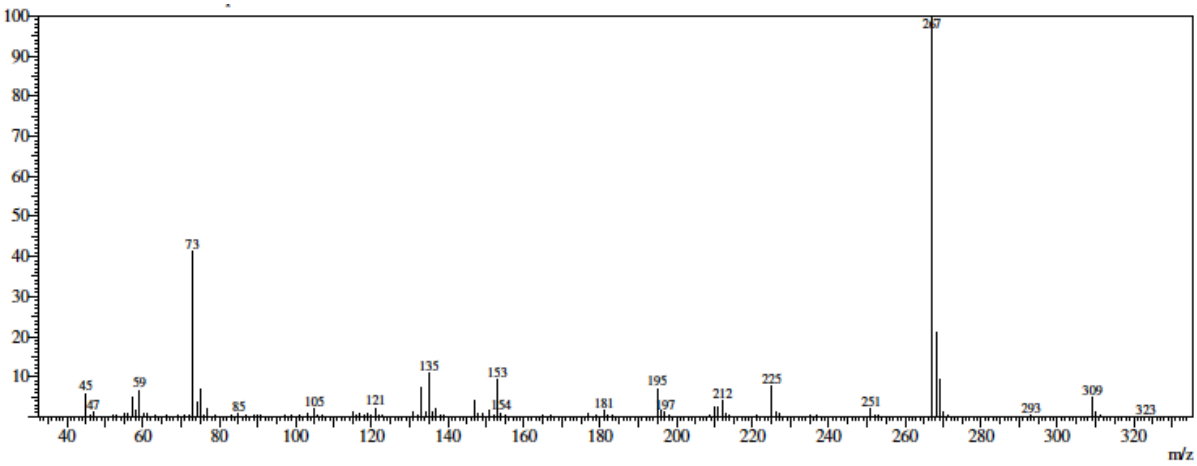


Figure 6: GC-MS chromatogram and mass spectrum for the detection of MPA derivatised using MTBSTFA. The retention time of 7.9minutes with m/z 267.

Qualitative Analysis of two phase derivatisation

The efficiency of the derivatisation reaction is assessed by determining the concentration of the unreacted MPA in the aqueous layer. Initial assessment of the efficiency of the reaction resulted in poor results. All three samples, as well as the three 200 ppm control standards, achieved higher peak areas than that of the 200 ppm standards used to construct the calibration curve. The MPA samples also had larger peak areas than 200ppm control standards that were made from the same stock solution. We should assume that if the derivatisation reaction was successful, the concentration of the aqueous layer should theoretically be less than the 200 ppm control standard. Concentrations were calculated using the equation from the initial calibration curve because it had the highest R^2 value.

%RSD for all the solutions were under 8 % which suggests good reproducibility however relative to the RSD values calculated for calibrations curves, the values are a lot higher.

Table 7: Quantitative results of un-reacted MPA and controls

	Sample Name					
	MPA 1	**MPA 2**	**MPA 3**	**Control 1**	**Control 2**	**Control 3**
Peak Area From Each	22557925	20083519	22001649	20964787	20237002	18626849

Triplicate Run	19653418	20813580	20264693	19065189	19237532	18818754
	20221916	22844236	19350322	18424502	18424502	19504924
Average	20811086	21247111	20542221	19499335	19299345	18983842
% RSD	7.39	6.73	6.53	6.69	4.70	2.42
Calculated Concentration (ppm)	**234.00**	**238.90**	**230.98**	**219.25**	**217.00**	**213.46**

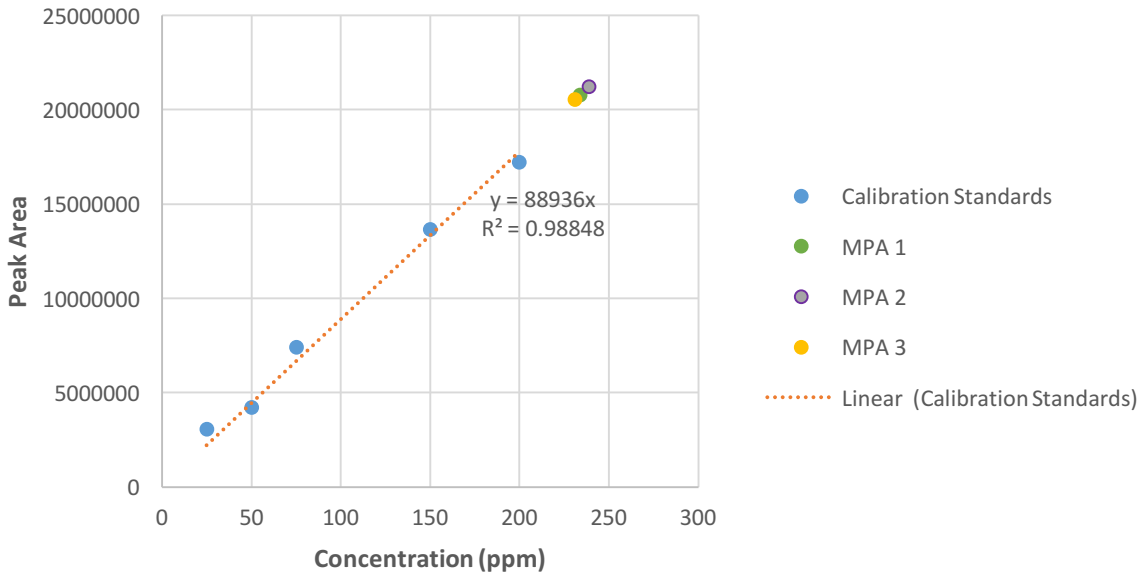


Figure 11: Sample MPA concentrations relative to the initial calibration curve (Figure 7).

A possible explanation for this increase in peak area was due to using a new 1000 ppm stock solution instead of the original 1000 ppm stock solution that was used to construct the calibration curve. The new stock solution was more concentrated by approximately 20 ppm (1084 ppm compared to 1064 ppm) which was approximately the difference between the 200 ppm calibration standard and the 200 ppm control standard. The increase in concentration for the analytes could also be due to the evaporation of water during the derivatisation reaction. Evaporation would decrease the volume of the aqueous layer thus concentrating the MPA. A solution to minimize this issue is to reconstitute the MPA solution back to 1 mL using de-

ionised water prior to the analysis. Quality controls would have to be made using the 200 ppm standard with the same volume extracted from the aqueous layer and made up using de-ionised water. This was not conducted due to time constraints.

The reproducibility of the peak areas (bar the 100 ppm calibration standards) supports MPA being a stable molecule. MPA in an aqueous environment is non-volatile with a half life of 18 years and highly resistant to most forms of chemical degradation [7]. There was no decrease in peak area that was observed in Chua's study [17]. Each run of the qualitative assessment for the unreacted MPA in the aqueous was conducted approximately 30 minutes apart with no obvious decrease in peak area. Control standards had also exhibited the same observations. Chua suggested that MPA adsorption onto the glassware could have been a possible cause for the large decrease in peak area. Subramaniam also supports this theory specifying that by using silylated glassware to reduce adsorption, increased the yield by MPA by 20 %. Other studies that address this phenomenon however, suggests that the loss of sample due to adsorption is small enough to consider it insignificant. %RSD were low across all samples, but further optimization of the LC-MS method could possibly further reduce these values.

CONCLUSION

Methylphosphonic acid was successfully derivatised using the two phase derivatisation method that was developed by Dival. The MPA derivative was able to be easily detected in the organic layer via GC-MS with defined peak shapes. A method to evaluate the efficiency of this reaction using LC-MS was then developed and optimized to give fast analysis times with good peak shape and resolution. Analysis using the developed method gave very reproducible results with a limit of detection and quantification values being as low as 0.134 ppm and 0.408 ppm respectively. Quantitative assessment of the derivatisation reaction was unsuccessful due to the concentrations of the un-reacted MPA in the aqueous layer were larger than control standards and the calibration standards. Poor sample preparation was the cause of the discrepancy between the 200 ppm control standards and the 200 ppm calibration standard. Increase in concentration of the analytes could be caused by evaporation of the aqueous sample thus concentrating the solution. Addressing these issues in future studies will assist in determining the efficiency of the proposed method by Dival.

References

[1] Vucinic S, Antonijevic B, Tsatsakis A. Environmental Exposure to Organophosphorus Nerve Agents. Environmental Toxicology and Pharmacology. 2017;56:163-71

[2] Black RM. History and perspectives of bioanalytical methods for chemical warfare agent detection. Journal of Chromatography B. 2010;878(17-18):1207 - 15.

[3] Hooijschuur E, Kientz C, Brinkman U. Analytical separation techniques for the determination of chemical warfare agents. Journal of Chromatograph A. 2002;982(2):177-200.

[4] Vale JA, Marrs T, Maynard R. Novichok: a murderous nerve agent attack in the UK. Clinical Toxicology. 2018;56(11):1093-7.

[5] Munro N, Talmage S. The Sources, Fate, and Toxicity of Chemical Warfare Agent Degradation Products. Environmental Health Perspectives. 1999;107(12):933-74.

[6] Seto Y, Tachikawa M, Kanamori-Kataoka M. Target Analysis of tert-butyldimethylsilyl Derivatives of Nerve Agent Hydrolysis Products by Selectable One-Dimensional or Two-Dimensional Gas Chromatography–Mass spectrometry. Journal of Chromatography A. 2017;1501:99-106.

[7] Mill T, Gould CW. Free-radical oxidation of organic phosphonic acid salts in water using hydrogen peroxide, oxygen, and ultraviolet light. Environmental Science & Technology. 1979;13(2):205-8.

[8] Read R, Black R. Rapid screening procedures for the hydrolysis products of chemical warfare agents using positive and negative ion liquid chromatography-mass spectrometry with atmospheric pressure chemical ionisation Journal of Chromatography A. 1999;862(2):169-77.

[9] Minami M, Hui D-M, Inagaki H. Method for the Analysis of the Methylphosphonic

Acid Metabolites of Sarin and its Ethanol-Substituted Analogue in Urine as Applied to the Victims of the Tokyo Sarin Disaster. Journal of Chromatography B: Biomedical Sciences and Applications 1997;695(2):237-44.

[10] Tripathi D, Pandey K, Bhattacharya A. Mass spectrometric identification of methyl phosphonic acid: the hydrolysis product of methyl phosphonofluoridate and pinacolyl methyl phosphonofluoridate Analytical Chemistry. 1992;64(72):823-34.

[11] Black R, Muir B. Derivatisation reactions in the chromatographic analysis of chemical warfare agents and their degradation products. Journal of Chromatography A. 2003;1000(1-2):253-81.

[12] Weissberg A, Madmon M. Determination of Organophosphorus Acids by Liquid Chromatography Positive Electrospray Ionization Tandem Mass Spectrometry after Chemical Derivatization. International Journal of Mass Spectrometry. 2016;408:20-7.

[13] D'Agostino P, Chenier C. Analysis of Chemical Warfare Agents: General Overview, LC-MS Review, In-House LC-ESI-MS Methods and Open Literature Bibliography. Canada: Defence Research and Development Canada; 2006.

[14] Read R, Black R. Rapid screening procedures for the hydrolysis products of chemical warfare agents using positive and negative ion liquid chromatography-mass spectrometry with atmospheric pressure chemical ionisation. Journal of Chromatography A. 1999;862(2):169-77.

[15] Black R, Read R. Analysis of degradation products of organophosphorus chemical warfare agents and related compounds by liquid chromatography-mass spectrometry using electrospray and atmospheric pressure chemical ionisation. Journal of Chromatography A. 1998;794(1-2):233-44.

[16] Dival L. The Development of an in-field Rapid Derivatisation Technique for the analysis of Chemical Warfare Agents Degradants [dissertation]. 2018.

[17] Chua X. Derivatisation of Chemical Warfare Agent Degradant Without Removal of Water [Project]. 2018.

[18] Liu Q, Hu X, Xie J. Determination of nerve agent degradation products in environmental samples by liquid chromatography–time-of-flight mass spectrometry with electrospray ionization. Analytica Chimica Acta. 2004;512(1):93-101.

[19] Baygildiev TM, Robin IA, Stavrianidi AN. Time-Efficient LC/MS/MS Determination of Low Concentrations of Methylphosphonic Acid. Inorganic Materials. 2017;53(14):1382-5.